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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [7] : C12Q 1/68	**A2**	**(11) International Publication Number:** **WO 00/58521** **(43) International Publication Date:** 5 October 2000 (05.10.00)

(21) International Application Number: PCT/US00/08604

(22) International Filing Date: 31 March 2000 (31.03.00)

(30) Priority Data:
60/127,223   31 March 1999 (31.03.99)   US

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(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

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(54) Title: METHODS FOR THE IDENTIFICATION OF REPORTER AND TARGET MOLECULES USING COMPREHENSIVE GENE EXPRESSION PROFILES

(57) Abstract

The present invention relates to methods of identifying genes whose expression is indicative of activation of a particular biochemical or metabolic pathway or a common set of biological reactions or functions in a cell ("regulon indicator genes"). The present invention provides an example of such an indicator gene. The present invention also relates to methods of partially characterizing a gene of unknown function by determining which biological pathways, reactions or functions its expression is associated with, thereby placing the gene within a functional genetic group or "regulon". These partially characterized genes may be used to identify desirable therapeutic targets of biological pathways of interest ("regulon target genes"). The present invention provides examples of such target genes. Methods for identifying effectors (activators and inhibitors) of regulon target genes are provided. The present invention also provides examples of regulon target gene inhibitors.

Methods for the Identification of Reporter and Target Molecules Using Comprehensive Gene Expression Profiles

TECHNICAL FIELD OF THE INVENTION

The present invention relates to methods of identifying genes whose expression is indicative of activation of a particular biochemical or metabolic pathway or a common set of biological reactions or functions in a cell ("regulon indicator genes"). The present invention provides an example of such an indicator gene. The present invention also relates to methods of partially characterizing a gene of unknown function by determining which biological pathways, reactions or functions its expression is associated with, thereby placing the gene within a functional genetic group or "regulon". These partially characterized genes may be used to identify desirable therapeutic targets of biological pathways of interest ("regulon target genes"). The present invention provides examples of such target genes. Methods for identifying effectors (activators and inhibitors) of regulon target genes are provided. The present invention also provides examples of regulon target gene inhibitors.

BACKGROUND OF THE INVENTION

The sequencing of the *S. cerevisiae* genome marked the first complete, ordered set of genes from a eukaryotic organism, and revealed the presence of over 6,000 genes on 16 chromosomes (Mewes et al., 1997, Goffeau et al., 1996). The DNA sequence revealed the presence of 6275 known and hypothetical open reading frames (ORFs) encoding putative proteins longer than 99 amino acids in length. Based upon codon usage, which can serve as a predictor of whether or not an ORF is actually expressed, there are currently thought to be 6222 expressed ORFs (Cherry et al., 1997).

The sequence of the roughly 6,000 ORFs in the yeast genome is compiled in the Saccharomyces Genome Database (SGD). The SGD provides Internet access to the complete genomic sequence of *S. cerevisiae*, ORFs, and the putative polypeptides encoded by these ORFs. The SGD can be accessed via the World Wide Web at http://genome-www.stanford.edu/Saccharomyces/ and http://www.mips.biochem.mpg.de/mips/yeast/. A gazetteer and genetic and physical maps of *S. cerevisiae* is found in Mewes et al., 1997 (incorporated herein by reference). References therein also contain the sequence of each chromosome of *S. cerevisiae* (incorporated herein by reference).

Having the complete DNA sequence of yeast available creates an opportunity to take a collectivist, rather than a reductionist, view on biology. We have developed a new technology that enables the simultaneous measurement of gene expression across an entire genome. The Genome Reporter Matrix™ (GRM) is a matrix of units comprising living yeast cells, the cells in each unit containing one yeast reporter fusion (GRM construct) representative of essentially every known hypothetical ORF of *S. cerevisiae*. See U.S. Pat. Nos. 5,569,588 and 5,777,888. A GRM construct comprises the promoter, 5' upstream untranslated region and usually the first four amino acids from one of each hypothetical ORF fused to a gene encoding an easily assayed reporter, such as green fluorescent protein (GFP), luciferin, or β-galactosidase. For a few GRM constructs, one to ten of the first amino acids from a hypothetical ORF is fused to the reporter. In addition, for those ORFs that have an intron, the entire first exon and the usually first four amino acids of the second exon are fused to the reporter. The GRM constructs are able to reveal changes in transcription for each hypothetical ORF in response to specific stimuli. In addition, the GRM constructs are able to reveal changes in mRNA splicing, translation and protein stability in those cases in which the N-terminus of the protein is sufficient for regulation.

The GRM provides an unprecedented view into the compensatory changes a cell makes in the face of a changing environment. Such environmental changes may be in the form of pH, salinity, temperature, osmotic pressure, nutrient

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availability, as well as biochemical perturbations caused by xenobiotics, pharmaceutical compounds and mutation. Identifying the compensatory changes a cell makes in response to exposure to a chemical can provide insight into the biological target of the chemical. For example, treatment of the GRM with the cholesterol-lowering drug lovastatin causes the cells to become depleted for sterols and non-sterol isoprenoids. The yeast cells respond by significantly up-regulating the genes encoding sterol biosynthetic enzymes and thus synthesizing more of the enzymes that make sterols. One may identify those genes that are involved in sterol biosynthesis or in related metabolic pathways by assaying the GRM. Because natural selection operates on a selected outcome rather than on a particular molecular mechanism, gene expression profiling strategies that detect regulatory changes through several molecular mechanisms contribute to a fuller view of how regulatory circuits have evolved.

An understanding of the regulatory circuits of yeast serves two purposes. On the one hand, yeast is an ideal model system for eukaryotic cells, including mammalian cells. Therefore, an understanding of the metabolic pathways of yeast can be used to design or discover drugs for use in plants and animals, including humans. On the other hand, yeast possess certain metabolic pathways and genes which are unique to yeast. An understanding of the differences between yeast and higher eukaryotes will permit the design and discovery of antifungal drugs that target genes and metabolic pathways specific to yeast. See U.S. Serial No. 60/127,272, filed concurrently herewith.

Yeast cells are eukaryotic and have many pathways that are similar or identical to those of mammalian cells. However, because yeast cells are unicellular, they are easier to manipulate experimentally and the results of such manipulations are easier to determine. Thus, yeast serves as an ideal model system for eukaryotic cells, including mammalian cells. The deduced protein sequences of the yeast genome display a significant amount of sequence identity with mammalian proteins. About one-third of the yeast ORFs, when aligned with their mammalian counterparts, produce a P-value score of less than 1×10^{-10} (Botstein et al., 1997). This number may in fact be a significant underestimate because the alignments were done with GenBank entries

that make up only about 10-20% of the unique human protein sequences thought to exist.

The evolutionary conservation between yeast and humans is not limited to sequence identity. The list of human genes that can functionally substitute for their yeast counterparts is extensive. For example, H-Ras (Kataoka et al., 1985), HMG-CoA reductase (Basson et al., 1988) and the heme A:farnesyltransferase (Glerum and Tzagoloff, 1994) have been shown to functionally replace their yeast counterparts. Researchers have utilized this evolutionary conservation to clone mammalian genes through their ability to complement the corresponding yeast mutants. Two examples include *CDC2* (Lee and Nurse, 1987) and *CDK2* (Elledge and Spottswood, 1991).

Functional conservation between yeast and humans may be best illustrated by the notable lack of antifungal therapeutic agents available for safely treating systemic infections in humans. Antifungal agents certainly exist, but they are characterized by profound side effects likely caused by inhibition of the mammalian counterparts of the yeast target. L659,699, lovastatin, and zaragozic acid inhibit different steps in the yeast sterol pathway (HMG-CoA synthase, HMG-CoA reductase, and squalene synthase, respectively). These inhibitors are also potent inhibitors of the corresponding mammalian enzymes (Correll and Edwards, 1994). In addition, we have found that in experiments with over 100 pharmaceutical agents used to treat a variety of distinct clinical indications in mammals, approximately 80% produced significant changes in gene expression in the GRM, indicating that there is substantial overlap in drug specificity between mammalian and yeast systems.

Yeast also contain genes that encode proteins that do not have plant and/or animal homologs. These non-homologous genes may be used as targets for the design and discovery of highly specific antifungal agents for use in plants and animals, including humans. The GRM may be used to identify genes that are expressed in particular metabolic pathways. Non-homologous genes in a pathway of interest may be used as targets for design and discovery of antifungal agents, for instance. See, e.g., U.S. Serial No. 60/127,272, filed concurrently herewith.

One metabolic pathway of interest for identification of both

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homologous and non-homologous genes is the pathway for synthesis of isoprenoids. Eukaryotic cells utilize a group of structurally related compounds, the isoprenoids, for a vast array of cellular processes. These processes include structural composition of the lipid bilayer, electron transport during respiration, protein glycosylation, tRNA

5   modification, and protein prenylation. All isoprenoids are synthesized via a pathway known variously as the isoprenoid pathway, mevalonate pathway, or sterol biosynthetic pathway. Although the bulk end product of the pathway is sterols, there are several branches of the pathway that lead to non-sterol isoprenoids. Due to the involvement of isoprenoids in a variety of physiologically and medically important

10   processes, a comprehensive understanding of the regulation of this pathway would offer many scientific and practical benefits.

The regulation of the isoprenoid biosynthetic pathway is known to be complex in all eukaryotic organisms examined, including *S. cerevisiae*. The overriding principle for the regulation of this pathway is multiple levels of feedback inhibition.

15   This feedback regulation is keyed to multiple intermediates and appears to act at numerous steps of the pathway, involving changes in transcription, translation and protein stability. Additionally, the availability of molecular oxygen, required for sterol and heme biosynthesis, also regulates the expression of genes at key steps of the pathway. The emerging picture is that the isoprenoid pathway has numerous points of

20   regulation that act to control overall flux through the pathway as well as the relative flux through various branches of the pathway.

Given the complexity of the isoprenoid pathway, it can be difficult to understand the regulation of any one step of this pathway, unless it is viewed within the context of the entire pathway. Thus, the GRM is ideal for understanding the

25   regulation of the isoprenoid pathway because one may observe the regulation of all the yeast genes involved in the isoprenoid pathway at one time by using the GRM. In addition, analysis of the gene expression provided by the GRM (preferably using software described below) may provide information about which particular genes in the isoprenoid pathway are important regulatory genes in the pathway, those which are

30   important indicator genes of the isoprenoid pathway, and those which are suitable

targets to regulate isoprenoid synthesis.

Today we have the luxury of reflecting upon the wealth of information that has come from decades of research into the cell biology and genetics of yeast. Still, less than 20% of the hypothetical ORFs discovered by the yeast genome project

5   had been previously identified through basic research (Goffeau et al., 1996). Additionally, 25% of the yeast ORFs with obvious human homologs have no known function (Botstein et al., 1997). The situation will likely be the same when the human genome sequence is completed.

Several research groups have created software programs that enable the

10   comparison of both chemical and genetic expression profiles to identify related gene expression response patterns, as shown, for example, in Figure 38. In addition, expression changes of individual genes in response to any given treatment can often be accessed through hypertext links. Currently, our software will: 1) normalize expression data; 2) rank changes in individual gene's expression relative to a particular

15   treatment; 3) rank similarities between genomic expression profiles as a result of a chemical or genetic treatment; and 4) determine the correlation coefficient for an individual gene's expression relative to that of all other genes to identify regulons, or groups of genes that share the same regulatory programs. See United States Application 09/076,668, now pending; Eisen et al. (1998); and Tamayo et al. (1999).

20   The ability to assign ORFs to functional groups based upon their expression patterns will provide valuable information pertaining to the function of proteins from model organisms as well as their mammalian counterparts. Analysis of genomic expression patterns may also reveal upstream regulatory sequences, including promoters, with great utility for regulated or constituitive expression of recombinant

25   genes. Such regulated sequences can be used for making reporter constructs for any selected process intrinsic to a given genome.

These functional genomics studies will provide a great deal of information that can implicate yeast genes, as well as their mammalian counterparts, in a variety of cellular functions. Associations of particular genes with specific biological

30   pathways will be made by virtue of the genes' patterns of regulation under numerous

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conditions.

One particular problem in the prior art has been identifying genes whose expression is representative of a specific biological (e.g., metabolic) pathway. One would like to be able to measure the expression of a gene or its encoded protein to

5   indicate the effect of a particular treatment on a specific pathway. Thus, there is a need for various pathway indicator genes for the various metabolic pathways.

A second problem in the prior art has been identifying genes and their encoded proteins which can be efficient targets within a specific biochemical pathway or set of associated pathways. Once good targets have been identified, pharmaceutical

10   compounds and treatments may be designed or discovered to regulate the expression or activity of the target gene or protein.

SUMMARY OF THE INVENTION

The instant invention addresses the above problems by providing a method using genomic arrays, such as the GRM or hybridization arrays, for identifying

15   indicator genes that are specific for particular biochemical pathways and sensitive to perturbations of these pathways. The instant invention provides one such gene, *HES1*, which is an indicator for the isoprenoid metabolic pathway. The invention provides the polynucleotide sequence of *HES1* and vectors and host cells comprising this sequence. The invention also provides a method of producing *HES1* recombinantly. The

20   invention further provides methods of using *HES1* as a specific indicator of the state of the isoprenoid pathway to identify compounds that regulate that pathway.

The instant invention also provides a method for identifying targets for one or more biochemical pathways of interest using the GRM or other types of genomic arrays, such as hybridization arrays. The instant invention also provides a

25   number of ORFs and their encoded proteins which are targets for lipid metabolism, yeast morphology, RNA metabolism and growth control. These ORFs include *YMR134w, YER034w, YJL105w, YKL077w, YGR046w, YJR041c, YER044c* and *YLR100w* and their encoded proteins.

The invention provides the polynucleotide sequences of these ORFs and

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vectors and host cells comprising these ORFs for use in methods of identifying, designing and discovering highly specific anti-target agents. Specific anti-target agents include antisense nucleic acid molecules that target *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and ribozymes that cleave

5   RNAs encoded by these ORFs. The invention also provides a methods of recombinantly producing the protein encoded by these ORFs for use as a target in methods of identifying, designing and discovering highly specific antifungal agents and for producing antibodies directed against the encoded protein. Specific anti-target agents include antibodies that bind to the protein encoded by *YMR134w*, *YER034w*,

10   *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and small organic molecules that bind to and inhibit proteins encoded by these ORFs.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Summary of Characteristics for *YJL105w*.

Figure 2. Plot of changes in expression of *YJL105w* and *CYB5* in

15   response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. *CYB5* functions in sterol biosynthesis through its activation of the Erg11p NADPH-cytochrome P-450 reductase.

20   **Figure 3.** Regulated Expression of *YJL105w*. *YJL105w* is significantly induced by isoprenoid biosynthetic inhibitors and mutations in HMG-CoA synthase (hmgs). "Log Ratio" refers to the natural log ratio of treated/untreated expression values.

                        Figure 4. Effects of lovastatin on wild-type and YJL105w knockout

25   yeast strains. 10 μl of a 25 mg/ml solution of lovastatin (250 μg) in ethanol was applied to a sterile drug disk on a lawn of yeast (5 x 10^6 cells, ABY363). The plates were incubated overnight at 30°C.

                        Figure 5. Summary of Characteristics for *YMR134w*.

                        Figure 6. Plot of changes in expression of *YMR134w* and *ERG2* in

response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. *ERG2* encodes sterol isomerase.

Figure 7. Treatments Causing Highest Expression of *YMR134w*.

YMR134w is induced most significantly by inhibitors of the isprenoid biosynthetic pathway.

Figure 8. Database Searches with *YMR134w*. Database searches with *YMR134w* did not reveal any apparent mammalian counterparts.

Figure 9. Summary of Characteristics for *YER044c*.

Figure 10. Plot of changes in expression of *YER044c* and *ERG2* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression.

Figure 11. Treatments Causing Highest Expression of *YER044c*.

YER044c is induced most significantly by inhibitors of the isprenoid biosynthetic pathway.

Figure 12. Database Searches with *YER044c*. Database searches with *YER044c* reveal numerous mammalian expressed-sequence tag (EST) apparent counterparts.

Figure 13. Comparison of the *YER044c* Predicted Protein Sequence with Mouse and Human EST Translations.

Figure 14. Comparison of the *YER044c* Predicted Protein Sequence with Rat EST Translation.

Figure 15. Summary of Characteristics for *YLR100w*.

Figure 16. Plot of changes in expression of *YLR100w* and *CYB5* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression.

Figure 17. Treatments Causing Highest Expression of *YLR100w*.

YLR100w is induced most significantly by inhibitors of isprenoid biosynthesis and a

mutation in the gene encoding Erg11p.

   Figure 18. Database Searches with *YLR100w*. Database searches with *YLR100w* reveal numerous mammalian expressed-sequence tag (EST) apparent counterparts.

   Figure 19. Alignment of *YLR100w* to Mammalian ESTs.

   Figure 20. Summary of Characteristics for *YER034w*.

   Figure 21. Plot of changes in expression of *YER034w* and *GPA2* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. Gpa2p, encoded by *GPA2*, is the alpha subunit of a trimer G-protein involved in pseudohyphal growth.

   Figure 22. Mutation of the *YER034w* Gene Leads to Increased Pseudohyphal Growth. Cells were plated onto low nitrogen plates (0.5% agarose, 2% glucose, 0.34% yeast nitrogen base without amino acids and ammonium sulfate, 0.05mM ammonium sulfate, 20 µg/ml uracil, 30 µg/ml leucine, and 5 µg/ml histidine) and incubated for four days at 25°C. Bar height represents the average number of hyphal projections per colony (n=20).

   Figure 23. Summary of Characteristics for *YKL077w*.

   Figure 24. Plot of changes in expression of *YKL077w* and *SGV1* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. *SGV1* is a Cdc28p-related protein kinase that is essential for yeast viability.

   Figure 25. Expression Correlation of *YKL077w*. Expression of the *YKL077w* gene correlates with that of genes involved in cell wall integrity and cytoskeletal reorganization.

   Figure 26. Database Searches with *YKL077w*. Database searches with *YKL077w* did not reveal any apparent mammalian counterparts.

   Figure 27. Summary of Characteristics for *YGR046w*.

   Figure 28. Plot of changes in expression of *YGR046w* and *IRA2* in

response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. *IRA2* encodes a GTPase-activating protein for Ras1p and Ras2p.

Figure 29. Expression Correlation of *YGR046w*. Expression of the *YGR046w* gene is correlated to other genes involved in growth control.

Figure 30. Treatments Causing the Most Significant Changes in Expression of *YGR046w*. Expression of *YGR046w* is sensitive to agents that perturb mitrochondrial function, create oxidative stress and disrupt the cytoskeleton.

Figure 31. Summary of Characteristics for *YJR041c*.

Figure 32. Plot of changes in expression of *YJR041c* and *MED7* in response to different chemical treatments. Each point represents the expression changes in a given chemical treatment. The fitness of the points to a line provides an indication of the level of coordinate gene expression. *MED7* is a component of the mediator complex involved in RNA Polymerase II transcription.

Figure 33. Expression Correlation of *YJR041c*. Expression of *YJR041c* is correlated to genes involved in RNA metabolism including RNA polymerase 1 and II transcription, mRNA splicing and turnover and ribosome function.

Figure 34. Database Searches with *YJR041c*. Database searches with *YJR041c* did not reveal any apparent mammalian counterparts.

Figure 35. Summary of Characteristics for *HES1*.

Figure 36. Expression Correlation of *HES1*.

Figure 37. Treatments that Induce the *HES1* Reporter. Inhibitors of the isoprenoid biosynthetic pathway cause a significant induction of the *HES1* reporter.

Figure 38. Browser Interface of Acacia's Expression Software.

Figure 39. *YJL105w* DNA Sequence.

Figure 40. *YJL105w* Protein Sequence.

Figure 41. *YMR134w* DNA Sequence.

Figure 42. *YMR134w* Protein Sequence.

Figure 43. *YER044c* DNA Sequence.

Figure 72. *YGL198w* DNA Sequence.

Figure 73. *YGL198w* Protein Sequence.

Figure 74. Each dot on the 4-quadrant plot represents a treatment affecting the reporters affecting *DAK1* and *PGU1*. Treatments are plotted as to whether *DAK1* was up-regulated (above x-axis) or down-regulated (below x-axis) and whether *PGU1* was up-regulated (right of the y-axis) or down-regulated (left of the y-axis). Thus, conditions where both reporters are up-regulated are in the upper right quadrant. Each division on the graph represents one natural log ratio change relative to controls. The *hog1* knock-out profile is indicated at the lower right. Thus, simultaneously measuring induction of *PGU1* above 2 natural log ratios and repression of *DAK1* below one natural ratio specifically indicates Hog1p pathway inactivation.

Figure 75. The plot description is the same as for **Figure 74**. The subset of treatments that target mitochondrial function form a distinct group in the upper right quadrant (within rectangle). Thus, simultaneously measuring induction of *YGL198w* and *STE18* should specifically indicate perturbations of the mitochondria.

DETAILED DESCRIPTION OF THE INVENTION

Definitions and General Techniques

Unless otherwise defined, all technical and scientific terms used herein have the meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics and immunology. See, e.g., Maniatis et al., 1982; Sambrook et al., 1989; Ausubel et al., 1992; Glover, 1985; Anand, 1992; Guthrie and Fink, 1991 (which are incorporated herein by reference).

A "regulon" is a group of genes that are coordinately regulated in response to a number of different stimuli, e.g., treatment with chemical compounds or mutations. The member genes of a regulon comprise a functional unit by which a cell is able to adapt to a changing environment. The regulation of these genes that led to their categorization could be at the level of transcription, mRNA stability, splicing,

translation or protein stability. The mode of regulation of each member gene of a given regulon need not be the same.

Genes are categorized into separate regulons based upon changes in gene expression. In order to efficiently and accurately group genes into functional groups, it is necessary to observe each gene's expression change. Since many genes function in specialized roles, it is necessary to measure global gene expression under as diverse a variety of conditions as possible. Therefore, the database of expression profiles used in this invention was made from a diverse collection of chemicals and mutant strains of yeast. In general, the greater the number of diverse stimuli which cause the genes of a regulon to exhibit coordinate expression and the higher the correlation coefficient, the more confident one will be that the regulon is a robust indicator of the pathway or process of interest.

A "regulon indicator gene" (RIG) is a gene whose expression changes when a particular regulon or biochemical pathway or cellular process is activated or repressed. Although a RIG's expression may correlate with a particular biochemical pathway, the RIG does not necessarily have to be a part of the biochemical pathway for which it is an indicator. A RIG may comprise the entire gene, the 5' region of the gene including the promoter and/or enhancer and all or a part of the coding region, or a fragment, conservatively modified variant or homolog thereof which retains the indicator function of the RIG. A RIG may be coordinately expressed with a particular biological pathway, such that when the pathway is activated the RIG is more highly expressed and when the pathway is repressed the RIG's expression is repressed as well. However, the invention also encompasses RIGs in which there is an inverse correlation with a particular pathway. In this case, activation of a pathway would lead to a repression of RIG expression, while repression of a pathway would lead to activation of RIG expression. A RIG may be coordinately expressed with a particular biological pathway, such that when the pathway is activated the RIG is more highly expressed. However, the invention also encompasses RIGs in which there is an inverse correlation with a particular pathway. In this case, activation of a pathway would lead to a repression of RIG expression. Furthermore, the invention also encompasses RIGs

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which are not necessarily part of the regulon, pathway or process for which they are indicators. In this case, expression of RIGs may be activated or repressed specifically in response to perturbations of a regulon, pathway or process even though the RIG itself may only be indirectly related or have no apparent relationship in function to the

5   regulon, pathway or process.

In a preferred embodiment, a RIG is specific to a particular pathway, wherein its expression changes most significantly when a particular pathway is activated or repressed. Such a highly specific regulon indicator gene cannot always be found for a pathway of interest. In such cases, more than one RIG can be identified

10   that, when their expression patterns are taken together, correlate with specificity to the pathway of interest. Thus, in another preferred embodiment, a plurality of RIGs is identified wherein the coordinated expression pattern of the plurality of RIGs is specific to a particular biological pathway. In this preferred embodiment, expression of each member of the plurality of RIGs may independently increase or decrease when the

15   biological pathway of interest is activated or repressed.

In another preferred embodiment, a RIG is highly sensitive to changes in activation or repression of a pathway, such that even a small perturbation in regulation of a pathway results in a change in RIG expression. In a further preferred embodiment, a RIG has a large dynamic range, and is highly induced or repressed upon

20   the corresponding perturbation of the pathway to which it is correlated.

In another preferred embodiment, a RIG does not contain sequences that are problematic for maintaining on plasmids when introduced into host cells. Such sequences that may be problematic include centromeric sequences or sites that are particularly susceptible to recombination.

25   A "target gene" or "regulon target gene" is a gene whose function is desirable to modulate. A target gene may consist of the entire gene, the 5' region comprising the promoter and/or enhancer and all or a part of the coding region, or a fragment, conservatively modified variant or homolog thereof which retains the function of the target gene. In general, a target gene encodes a protein which is a part

30   of the biological (e.g., metabolic or biochemical) pathway or process whose

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modulation would result in a desired outcome. In a preferred embodiment, a target gene is a control point in such a pathway. In one more preferred embodiment, a target gene is a control point that is relatively "upstream" in the metabolic pathway. "Upstream" means that the target gene is involved in one of the first steps of the

5   metabolic pathway or process. In another more preferred embodiment, a target gene is a control point that is relatively "downstream" but specific to a biological pathway or a branch of that pathway or process. "Downstream" means that the target gene is involved in one of the later steps of the pathway or process.

A "target" or "target protein" is a protein whose expression or activity

10   is to be modulated. A target may consist of the entire protein or a fragment, mutein, derivative or homolog thereof which retains the function of the target. In general, a target is a protein included within a biological pathway wherein it is desired to modulate the process which the protein is involved in. In a preferred embodiment, a target is a control point in such a biological pathway. In a more preferred

15   embodiment, a target is a control point that is relatively "upstream" in the biological pathway. "Upstream" means that the target is involved in one of the first steps of the pathway. In another more preferred embodiment, a target is a control point that is relatively "downstream" but specific to a biological pathway or a branch of that pathway. "Downstream" means that the target is involved in one of the later steps of

20   the pathway.

A "target-dependent reporter gene" is a gene whose expression is altered in a cell in which the target gene has been altered or inactivated compared to the cell which expresses the normal target gene. The expression of the target-dependent reporter gene may increase or decrease in a cell harboring an altered or

25   inactivated target gene, depending upon the identity of the gene. If expression of the target-dependent reporter gene increases in the cell harboring the altered or inactivated target gene, then a potential inhibitor of the regulon target gene will increase expression of the target-dependent reporter gene, and if expression of the target-dependent reporter gene decreases in the cell, then a potential inhibitor of the regulon

30   target gene will decrease expression of the target-dependent reporter gene.

By "pathway" is meant any biological, e.g., metabolic or biochemical, set of concerted reactions which occur in response to a particular signal or stimulus in a cell. The isoprenoid pathway is one example of such a pathway. Other pathways include, without limitation, amino acid and protein synthesis, lipid synthesis, protein

5   and lipid glycosylation, protein modification, DNA synthesis and repair, RNA transcription, phospholipid synthesis, nucleotide synthesis, and energy generation and storage (e.g., glycolysis, citric acid cycle, oxidative phosphorylation, gluconeogenesis, pentose phosphate pathway, fatty acid metabolism, glycogen and disaccharide metabolism, amino acid degradation and the urea cycle), signal transduction and

10   growth control.

By "process" is meant any biological reaction or set of reactions that occurs within a cell or organism that occurs in response to a stimulus or signal, or that occurs during growth, homeostasis, development, differentiation or death of the cell or organism.

15   An "isolated" protein or polypeptide is one that has been separated from naturally associated components that accompany it in its native state. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally

20   associated components by isolation, using protein purification techniques well known in the art.

A monomeric protein is "substantially pure," "substantially homogeneous" or "substantially purified" when at least about 60 to 75% of a sample exhibits a single polypeptide sequence. A substantially pure protein will typically

25   comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis of a protein sample, followed by visualizing a single polypeptide band upon staining the gel with a stain well known in the art. For certain purposes, higher resolution may be

30   provided by using HPLC or other means well known in the art for purification.

A *S. cerevisiae* protein has "homology" or is "homologous" to a protein from another organism if the encoded amino acid sequence of the yeast protein has a similar sequence to the encoded amino acid sequence of a protein of a different organism. Alternatively, a *S. cerevisiae* protein may have homology or be homologous to another *S. cerevisiae* protein if the two proteins have similar amino acid sequences. Although two proteins are said to be "homologous," this does not imply that there is necessarily an evolutionary relationship between the proteins. Instead, the term "homologous" is defined to mean that the two proteins have similar amino acid sequences. In addition, although in many cases proteins with similar amino acid sequences will have similar functions, the term "homologous" does not imply that the proteins must be functionally similar to each other.

When "homologous" is used in reference to proteins or peptides, it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent sequence identity or degree of homology may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art (see, e.g., Pearson et al.,1994, and [Henikoff et al., 1992, herein incorporated by reference).

The following six groups each contain amino acids that are conservative substitutions for one another:

1)   Alanine (A), Serine (S), Threonine (T);

2)   Aspartic Acid (D), Glutamic Acid (E);

3)   Asparagine (N), Glutamine (Q);

4)   Arginine (R), Lysine (K);

5)   Isoleucine (I), Leucine (L), Methionine (M), Valine (V), and

6)   Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

Sequence homology for polypeptides, which is also referred to as sequence identity, is typically measured using sequence analysis software. See, e.g., the Sequence Analysis Software Package of the Genetics Computer Group (GCG), University of Wisconsin Biotechnology Center, 910 University Avenue, Madison, Wisconsin 53705. Protein analysis software matches similar sequences using measure of homology assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG contains programs such as "Gap" and "Bestfit" which can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof.

A preferred algorithm when comparing a *S. cerevisiae* sequence to a database containing a large number of sequences from different organisms is the computer program BLAST, especially blastp or tblastn (Altschul et al., 1997, herein incorporated by reference). Preferred parameters for blastp are:

Expectation value: 10 (default)
Filter: seg (default)
Cost to open a gap: 11 (default)
Cost to extend a gap: 1 (default
Max. alignments: 100 (default)
Word size: 11 (default)
No. of descriptions: 100 (default)
Substitution Matrix: BLOSUM62

The length of polypeptide sequences compared for homology will generally be at least about 16 amino acid residues, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues. When searching a database containing sequences from a large number of different organisms using a *S. cerevisiae* query sequence, it is preferable to compare amino acid sequences. Comparison of amino acid sequences is preferred to comparing nucleotide sequences because *S. cerevisiae* has significantly different codon usage compared to mammalian or plant codon usage.

Database searching using amino acid sequences can be measured by

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algorithms other than blastp known in the art. For instance, polypeptide sequences can be compared using Fasta, a program in GCG Version 6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, 1990, herein incorporated by reference). For example,

5   percent sequence identity between amino acid sequences can be determined using Fasta with its default parameters (a word size of 2 and the PAM250 scoring matrix), as provided in GCG Version 6.1, herein incorporated by reference.

The invention envisions two general types of polypeptide "homologs." Type 1 homologs are strong homologs. A comparison of two polypeptides that are

10   Type 1 homologs would result in a blastp score of less than 1×10^{-40}, using the blastp algorithm and the parameters listed above. The lower the blastp score, that is, the closer it is to zero, the better the match between the polypeptide sequences. For instance, yeast lanosterol demethylase, which is a common target of antifungal agents, as discussed above, has a Type 1 homolog in humans. The probability score (e.g.,

15   blastp score) is dependent upon the size of the database. Comparison of yeast and human lanosterol demethylases produces a blastp score of 1×10^{-86}.

Type 2 homologs are weaker homologs. A comparison of two polypeptides that are Type 2 homologs would result in a blastp score of between 1×10^{-40} and 1×10^{-10}, using the Blast algorithm and the parameters listed above. One having

20   ordinary skill in the art will recognize that other algorithms can be used to determine weak or strong homology.

The terms "no substantial homology" or "no human (or mammalian, vertebrate, amphibian, fish, insect or plant) homolog" refers to a yeast polypeptide sequence which exhibits no substantial sequence identity with a polypeptide sequence

25   from human, non-human mammals, other vertebrates, insects or plants. A comparison of two polypeptides which have no substantial homology to one another would result in a blastp score of greater than 1×10^{-10}, using the Blast algorithm and the parameters listed above. One having ordinary skill in the art will recognize that other algorithms can be used to determine whether two polypeptides demonstrate no substantial

30   homology to each other.

20

A polypeptide "fragment," "portion" or "segment" refers to a stretch of amino acid residues of at least about five to seven contiguous amino acids, often at least about seven to nine contiguous amino acids, typically at least about nine to 13 contiguous amino acids and, most preferably, at least about 20 to 30 or more

5   contiguous amino acids.

A polypeptide "mutein" refers to a polypeptide whose sequence contains substitutions, insertions or deletions of one or more amino acids compared to the amino acid sequence of the native or wild type protein. A mutein has at least 50% sequence homology to the wild type protein, preferred is 60% sequence homology,

10   more preferred is 70% sequence homology. Most preferred are muteins having 80%, 90% or 95% sequence homology to the wild type protein, in which sequence homology is measured by any common sequence analysis algorithm, such as Gap or Bestfit.

A "derivative" refers to polypeptides or fragments thereof that are

15   substantially homologous in primary structural sequence but which include, e.g., *in vivo* or *in vitro* chemical and biochemical modifications or which incorporate unusual amino acids. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labeling, e.g., with radionuclides, and various enzymatic modifications, as will be readily appreciated by those well skilled in

20   the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the art, and include radioactive isotopes such as ^{125}I, ^{32}P, ^{35}S, and ^{3}H, ligands which bind to labeled antiligands (e.g., antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label

25   depends on the sensitivity required, ease of conjugation with the primer, stability requirements, and available instrumentation. Methods for labeling polypeptides are well known in the art. See Ausubel et al., 1992, hereby incorporated by reference.

The term "fusion protein" refers to polypeptides comprising polypeptides or fragments coupled to heterologous amino acid sequences. Fusion

30   proteins are useful because they can be constructed to contain two or more desired

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functional elements from two or more different proteins. Fusion proteins can be produced recombinantly by constructing a nucleic acid sequence which encodes the polypeptide or a fragment thereof in frame with a nucleic acid sequence encoding a different protein or peptide and then expressing the fusion protein. Alternatively, a

5   fusion protein can be produced chemically by crosslinking the polypeptide or a fragment thereof to another protein.

An "isolated" or "substantially pure" nucleic acid or polynucleotide (e.g., an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components that naturally accompany the native polynucleotide in its

10   natural host cell, e.g., ribosomes, polymerases, or genomic sequences with which it is naturally associated. The term embraces a nucleic acid or polynucleotide that has been removed from its naturally occurring environment. The term "isolated" or "substantially pure" also can be used in reference to recombinant or cloned DNA isolates, chemically synthesized polynucleotide analogs, or polynucleotide analogs that

15   are biologically synthesized by heterologous systems.

The term "percent sequence identity" or "identical" in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over a stretch of at least about nine nucleotides, usually at least

20   about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using Fasta, a program in GCG Version

25   6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, 1990, herein incorporated by reference). For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta with its default parameters (a word size of 6 and the NOPAMfactor for the scoring matrix) as provided in GCG Version 6.1, herein

30   incorporated by reference.

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The term "substantial homology" or "substantial similarity," when
referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned
with appropriate nucleotide insertions or deletions with another nucleic acid (or its
complementary strand), there is nucleotide sequence identity in at least about 60% of

5   the nucleotide bases, usually at least about 70%, more usually at least about 80%,
preferably at least about 90%, and more preferably at least about 95-98% of the
nucleotide bases, as measured by any well-known algorithm of sequence identity, such
as Fasta, as discussed above.

Alternatively, substantial homology or similarity exists when a nucleic

10   acid or fragment thereof hybridizes to another nucleic acid, to a strand of another
nucleic acid, or to the complementary strand thereof, under selective hybridization
conditions. Typically, selective hybridization will occur when there is at least about
55% sequence identity -- preferably at least about 65%, more preferably at least about
75%, and most preferably at least about 90% -- over a stretch of at least about 14

15   nucleotides. See, e.g., Kanehisa, 1984, herein incorporated by reference.

Nucleic acid hybridization will be affected by such conditions as salt
concentration, temperature, solvents, the base composition of the hybridizing species,
length of the complementary regions, and the number of nucleotide base mismatches
between the hybridizing nucleic acids, as will be readily appreciated by those skilled in

20   the art. "Stringent hybridization conditions" and "stringent wash conditions" in the
context of nucleic acid hybridization experiments depend upon a number of different
physical parameters. The most important parameters include temperature of
hybridization, base composition of the nucleic acids, salt concentration and length of
the nucleic acid. One having ordinary skill in the art knows how to vary these

25   parameters to achieve a particular stringency of hybridization. In general, "stringent
hybridization" is performed at about 25°C below the thermal melting point (T_m) for the
specific DNA hybrid under a particular set of conditions. "Stringent washing" is
performed at temperatures about 5°C lower than the T_m for the specific DNA hybrid
under a particular set of conditions. The T_m is the temperature at which 50% of the

30   target sequence hybridizes to a perfectly matched probe. See Sambrook et al., page

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9.51, hereby incorporated by reference.

The T_m for a particular DNA-DNA hybrid can be estimated by the formula:

$T_m = 81.5°C + 16.6 (log_{10}[Na^+]) + 0.41 (fraction G + C) - 0.63 (\%$ formamide) - (600/l) where l is the length of the hybrid in base pairs.

The T_m for a particular RNA-RNA hybrid can be estimated by the formula:

$T_m = 79.8°C + 18.5 (log_{10}[Na^+]) + 0.58 (fraction G + C) + 11.8$ (fraction G + C)^2 - 0.35 (% formamide) - (820/l).

The T_m for a particular RNA-DNA hybrid can be estimated by the formula:

$T_m = 79.8°C + 18.5(log_{10}[Na^+]) + 0.58 (fraction G + C) + 11.8$ (fraction G + C)^2 - 0.50 (% formamide) - (820/l).

In general, the T_m decreases by 1-1.5°C for each 1% of mismatch between two nucleic acid sequences. Thus, one having ordinary skill in the art can alter hybridization and/or washing conditions to obtain sequences that have higher or lower degrees of sequence identity to the target nucleic acid. For instance, to obtain hybridizing nucleic acids that contain up to 10% mismatch from the target nucleic acid sequence, 10-15°C would be subtracted from the calculated T_m of a perfectly matched hybrid, and then the hybridization and washing temperatures adjusted accordingly. Probe sequences may also hybridize specifically to duplex DNA under certain conditions to form triplex or other higher order DNA complexes. The preparation of such probes and suitable hybridization conditions are well known in the art.

An example of stringent hybridization conditions for hybridization of complementary nucleic acid sequences having more than 100 complementary residues on a filter in a Southern or Northern blot or for screening a library is 50% formamide/6X SSC at 42°C for at least ten hours. Another example of stringent hybridization conditions is 6X SSC at 68°C for at least ten hours. An example of low stringency hybridization conditions for hybridization of complementary nucleic acid sequences having more than 100 complementary residues on a filter in a Southern or

northern blot or for screening a library is 6X SSC at 42°C for at least ten hours.
Hybridization conditions to identify nucleic acid sequences that are similar but not
identical can be identified by experimentally changing the hybridization temperature
from 68°C to 42°C while keeping the salt concentration constant (6X SSC), or

5   keeping the hybridization temperature and salt concentration constant (e.g. 42°C and
6X SSC) and varying the formamide concentration from 50% to 0%. Hybridization
buffers may also include blocking agents to lower background. These agents are well-
known in the art. See Sambrook et al., pages 8.46 and 9.46-9.58, herein incorporated
by reference.

10   Wash conditions also can be altered to change stringency conditions.
An example of stringent wash conditions is a 0.2x SSC wash at 65°C for 15 minutes
(see Sambrook et al., for SSC buffer). Often the high stringency wash is preceded by a
low stringency wash to remove excess probe. An exemplary medium stringency wash
for duplex DNA of more than 100 base pairs is 1x SSC at 45°C for 15 minutes. An

15   exemplary low stringency wash for such a duplex is 4x SSC at 40°C for 15 minutes.
In general, signal-to-noise ratio of 2x or higher than that observed for an unrelated
probe in the particular hybridization assay indicates detection of a specific
hybridization.

As defined herein, nucleic acids that do not hybridize to each other

20   under stringent conditions are still substantially homologous to one another if they
encode polypeptides that are substantially identical to each other. This occurs, for
example, when a nucleic acid is created synthetically or recombinantly using a high
codon degeneracy as permitted by the redundancy of the genetic code.

The polynucleotides of this invention may include both sense and

25   antisense strands of RNA, cDNA, genomic DNA, and synthetic forms and mixed
polymers of the above. They may be modified chemically or biochemically or may
contain non-natural or derivatized nucleotide bases, as will be readily appreciated by
those of skill in the art. Such modifications include, for example, labels, methylation,
substitution of one or more of the naturally occurring nucleotides with an analog,

30   internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates,

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phosphotriesters, phosphoramidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendent moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.) Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

"Conservatively modified variations" or "conservatively modified variants" of a particular nucleic acid sequence refers to nucleic acids that encode identical or essentially identical amino acid sequences or DNA sequences where no amino acid sequence is encoded. Due to the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide sequence. When a nucleic acid sequence is changed at one or more positions with no corresponding change in the amino acid sequence which it encodes, that mutation is called a "silent mutation." Thus, one species of a conservatively modified variation according to this invention is a silent mutation. Accordingly, every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent mutation or variation.

Furthermore, one of skill in the art will recognize that individual substitutions, deletions, additions and the like, which alter, add or delete a single amino acid or a small percentage of amino acids (less than 5%, more typically less than 1%) in an encoded sequence are "conservatively modified variations" or "conservatively modified variants" where the alterations result in the substitution of one amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The term "antibody" refers to a polypeptide encoded by an immunoglobulin gene, genes, or fragments thereof. The immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant regions, as well as a myriad of immunoglobulin variable regions. Light chains are classified as either kappa

or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes IgG, IgM, IgA, IgD and IgE, respectively.

Antibodies exist for example, as intact immunoglobulins or as a number of well-characterized fragments produced by digestion with various peptidases. For example, trypsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab)'_2$, a dimer of Fab which itself is a light chain joined to a V_H-C_H1 by a disulfide bond. The $F(ab)'_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the $F(ab)'_2$ dimer to a Fab' monomer. The Fab' monomer is essentially an Fab with part of the hinge region. See Paul (1993) (incorporated herein by reference), for a detailed description of epitopes, antibodies and antibody fragments. One of skill in the art recognizes that such Fab' fragments may be synthesized *de novo* either chemically or using recombinant DNA technology. Thus, as used herein, the term antibody includes antibody fragments produced by the modification of whole antibodies or those synthesized de novo. The term antibody also includes single-chain antibodies, which generally consist of the variable domain of a heavy chain linked to the variable domain of a light chain. The production of single-chain antibodies is well known in the art (see, e.g., U.S. Pat. No. 5,359,046). The antibodies of the present invention are optionally derived from libraries of recombinant antibodies in phage or similar vectors (see, e.g., Huse et al. (1989); Ward et al. (1989); Vaughan et al. (1996) which are incorporated herein by reference).

As used herein, "epitope" refers to an antigenic determinant of a polypeptide, i.e., a region of a polypeptide that provokes an immunological response in a host. This region need not comprise consecutive amino acids. The term epitope is also known in the art as "antigenic determinant." An epitope may comprise as few as three amino acids in a spatial conformation which is unique to the immune system of the host. Generally, an epitope consists of at least five such amino acids, and more usually consists of at least 8-10 such amino acids. Methods for determining the spatial conformation of such amino acids are known in the art.

Methods for Analyzing ORF Gene Expression

The cell's ability to monitor its own biochemical ecology may be considered as a fully integrated multi-dimensional set of specific biochemical assays. The data from each individual assay manifests itself either directly or indirectly in the change in expression of a single gene or small set of genes. The individual components of the assaying capabilities of the cell may be extracted by measuring the changes in global gene expression in response to a controlled experimental challenge.

The measurement of global gene expression may be done by a number of different methods. One technique is that of hybridization to nucleic acid arrays on solid surfaces, such as "gene chips" (Fodor et al., 1991). Another method uses a reporter construct in the GRM or an equivalent matrix comprising living cells, preferably eukaryotic cells, and more preferably yeast, insect, plant, avian, fish or mammalian cultured cells. Other methods include SAGE.

DNA Chip Technology

One method for determining comprehensive gene expression profiles is DNA gene chip technology (see, e.g., Fodor et al., 1991). A DNA gene chip can be made comprising a large number of immobilized single-stranded nucleic acids, each of which hybridizes specifically to a gene or its mRNA, representing a particular genome or a significant subset thereof. Messenger RNA molecules extracted from a cell or cDNA molecules converted from such mRNA molecules can be labeled. The labeling can be accomplished, for example, radioisotopically or fluorescently by methods well known in the art. These mRNA or cDNA molecules are rendered single-stranded and then allowed to hybridize to the immobilized single-stranded nucleic acids on the gene chip. A computer equipped with a scanner then determines the extent of hybridization, thereby quantitating the amount of mRNA produced for any given gene or genetic sequence.

Profiles of gene expression generated under different conditions or in response to different stimuli such as treatment with chemical compounds are produced by treating cells with a compound, isolating the mRNA the cells, optionally producing

cDNA and then hybridizing the single-stranded nucleic acids on the gene chip as discussed above. Preferably, software is used to correlate the expression of each gene on the hybridization chip relative to other genes under different conditions or in response to different treatments (see below).

Promoter elements from genes of interest that respond to an input signal can then be isolated and operatively linked to a reporter gene described above by recombinant DNA techniques well known in the art for further characterization.

Genome Reporter Matrix™ Technology

An alternative method to DNA gene chip technology is the use of a Genome Reporter Matrix™ (GRM), or an equivalent thereof. The description below of the generation of gene expression profiles utilizing the Genome Reporter Matrix™ has been described essentially in United States Patents 5,569,888 and 5,777,888, both of which are incorporated herein by reference.

The promoter (and optionally, 5' upstream regulatory elements and/or 5' upstream untranslated sequences) of an ORF or a gene from a cellular genome (preferably a eukaryotic genome) is fused to a reporter gene creating a transcriptional and/or translational fusion of the promoter to the reporter gene. In a preferred embodiment, the genome is that of *S. cerevisiae*. The promoter and optional additional sequences comprise all the regulatory elements necessary for transcriptional (and optionally translational) control of an attached coding sequence. The reporter gene can be any gene that, when expressed in a suitable host, encodes a product that can be detected by a quantitative assay. Any suitable assay may be used, including but not limited to enzymatic, colorimetric, fluorescence or other spectrographic assays, fluorescent activated cell sorting assay and immunological assays. Examples of suitable reporter genes include, inter alia, green fluorescent protein (GFP), β-lactamase, lacZ, invertase, membrane bound proteins (e.g., CD2, CD4, CD8, the influenza hemagglutinin protein, and others well known in the art) to which high affinity antibodies directed to them exist or can be made routinely, fusion protein comprising membrane bound protein appropriately fused to an antigen tag domain

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(e.g., hemagglutinin or Myc and others well known in the art). In a preferred embodiment, the reporter protein is GFP from the jellyfish *Aequorea victoria*. GFP is a naturally fluorescing protein that does not require the addition of any exogenous substrates for activity. The ability to measure GFP fluorescence in intact living cells

5   makes it an ideal reporter protein for the GRM or an equivalent matrix comprising living cells.

In a preferred embodiment, reporter constructs comprise the 5' region of the ORF comprising the promoter of the ORF and other expression regulatory sequences, and generally the first four codons of the ORF fused in-frame to the green

10 fluorescent protein. In a more preferred embodiment, approximately 1200 base-pairs of 5' regulatory sequence are included in each fusion. Only 228 yeast ORFs (3.5%) possess introns. Of these 228 intron-containing ORFs, all but four contain only one intron. In these ORFs, fusions are created two to four codons past (3' to) the splice junction. Therefore, these fusions must undergo splicing in order to create a functional

15 reporter fusion.

Each reporter is assembled in an episomal yeast shuttle vector (either CEN or 2μ plasmid) or on a yeast integrating vector for subsequent insertion into the chromosomal DNA. In a preferred embodiment, the gene reporter constructs are built using a yeast multicopy vector. A multicopy vector is chosen to facilitate easy transfer

20 of the reporter constructs to many different yeast strain backgrounds. In addition, the vector replicates at an average of 10 to 20 copies per cell, providing added sensitivity for detecting genes that are expressed at a low level. In principle, introducing additional copies of a gene's regulatory region could, through titration of regulatory proteins, disrupt a response of interest. However, in practice this appears not to occur,

25 and efforts to successfully exploit such titration effects have required much higher copy number vectors and have been largely unsuccessful. In another preferred embodiment, the reporter constructs are maintained on episomal plasmids in yeast.

In one embodiment, a plurality (all or a significant subset) of the resulting approximately 6,000 reporter constructs is transformed into a strain of yeast.

30 The resulting strains constitute one embodiment of the Genome Reporter Matrix™.

See Example 1.

Profiles are produced by arraying wild type or mutant cells carrying the reporter fusion genes in growth media containing different drugs and chemical compounds and measuring changes in expression of the reporter gene by the appropriate assay (see below). In a preferred embodiment, where the reporter gene is GFP, measurement of changes in expression are done by measuring the amount of green light produced by the cells over time with an automated fluorescence scanner. Alternatively, the drugs or chemical compounds may be added to the yeast cells after they have been arrayed onto growth media and then measuring changes in reporter gene expression by the appropriate assay.

Over 93% of the reporters are detectable over background on rich medium. The reproducibility of individual reporters is high, with expression generally varying by less than 10%. In contrast, hybridization experiments have proven unreliable for effects of less than a factor of two. **Figure 64** depicts expression data of the GRM from two independent experiments plotted against each other.

In a preferred embodiment, the GRM is used to obtain gene expression information from a genome. The GRM is preferred to hybridization-based methods of profiling for several reasons. First, because the promoter-reporter fusions include the first four amino acids of the native gene product, the response profiles are composites of both transcriptional and translational effects. The importance of being able to monitor both levels of response is underscored by the experience with bacterial antibiotics. Those antibiotics that work at the translational level have a greater therapeutic performance than those affecting transcription. Because hybridization-based methods can reveal only effects on transcription, profiling with the GRM provides a more complete view of the full spectrum of biological effects induced by exposure to drugs or compounds.

Second, the GRM permits profiling of gene expression changes in living cells, which permits one to easily measure the kinetics of changes in gene response profiles in the same population of cells following exposure to different drugs and

chemical agents. Thus, by collecting multiple data sets over time, one can identify the genes that make up primary and secondary responses.

Third, hybridization-based methods require relatively sophisticated molecular procedures to produce labeled cDNA, followed by a 14 hour hybridization

5   of labeled cDNA probes to target DNA arrays on slides or chips. The GRM requires only that being able to produce arrays of colonies and measure emitted light. These procedures are easier to scale up in an industrial setting than are sophisticated molecular biology methods, rendering data that is more straightforward to produce and more reproducible in nature.

10   *Gene Expression Profiles*

Using the reporter construct, gene chip technology or another method for obtaining genome-wide gene expression, the gene expression profile of yeast genes can be obtained. In a preferred embodiment, either the GRM or gene chip technology is used. In a more preferred embodiment, the GRM is treated with a number of

15   pharmaceutical compounds and the resulting expression of the reporter constructs is analyzed. Generally, for each pharmaceutical compound, the expression of the reporter constructs are analyzed in the presence of the vehicle for the pharmaceutical compound alone and is compared to the expression of the reporter constructs in the presence of the pharmaceutical compound. Changes in expression of the reporter

20   constructs in the absence and presence of the pharmaceutical compound is obtained either by subtracting the baseline level of expression from the level after treatment or dividing the baseline level of expression from the level after treatment. By looking at a large number of reporter constructs, one can assign yeast ORFs to functional groups based upon their expression patterns in response to various pharmaceutical

25   compounds. These functional groups may provide valuable information as to the function of the yeast proteins as well as their human, non-human mammalian, avian, fish, insect and plant counterparts.

Preferably, software is used to correlate the expression of each gene in the GRM or on the DNA chip relative to other genes under different conditions and in

response to different pharmaceutical compounds. In one preferred embodiment, the software is capable of producing a correlation coefficient for each gene's expression relative to every other gene across all expression profiles in a database. Such analysis reveals groups of genes that exhibit coordinate regulation (regulons). See, e.g., U.S. Serial No. 09/076,668, now pending; Eisen et al. (1998); and Tamayo et al. (1999).

In a preferred embodiment, a gene of unknown function may be placed into a functional genetic group by the following steps:

a)   generating a gene expression profile for Gene X, a gene of unknown function;

b)   comparing the gene expression profile of Gene X with expression profiles of a plurality of other genes in a database of compiled gene expression profiles to generate expression correlation coefficients;

c)   identifying based on their expression correlation coefficients a set of genes comprising Gene X that are coordinately expressed;

d)   determining if the genes whose expression is most highly correlated with that of Gene X belong to a gene regulon involved in a known biological pathway, or a common set of biological reactions or functions; and

e)   optionally testing the effect on Gene X expression of at least one altered condition or treatment known to affect the function to which Gene X hs been ascribed.

If Gene X expression is coordinate with expression of the regulon, then Gene X is placed in the regulon.

Methods to Identify Potential RIGs

A GRM (or an equivalent) is chemically treated with a large number of compounds. Regulons are identified as groups of genes that are coordinately regulated in response to genetic mutations, treatment with compounds or different environmental conditions. In a preferred embodiment, regulons are identified using correlation

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coefficients assembled by software that does clustering analysis, such as that described in U.S. Serial No. 09/076,668, now pending; Eisen et al. (1998); and Tamayo et al. (1999). In a preferred embodiment, genes that constitute a regulon have a correlation coefficient of greater than 0.5. In a more preferred embodiment, genes that constitute

5   a regulon have a correlation coefficient of at least 0.6 or 0.7. In a further preferred embodiment, genes that constitute a regulon have a correlation coefficient of at least 0.8 or 0.9. The correlation coefficient may be measured by any method of obtaining correlation coefficients, including, without limitation, the method described in United States Patent Application Serial No. 09/076,668, now pending or in Eisen et al.

10   (1998).

Once a group of genes has been grouped into a regulon, one can identify potential regulon indicator genes (RIGs), which may or may not be a member of the regulon, pathway or process with the regulon, pathway, or process for which they are an indicator. RIGs may be either characterized or uncharacterized genes

15   provided they have certain characteristics. Preferred characteristic include one or more of the following: 1) its expression profile is sensitive to one or more stimuli; 2) its expression profile exhibits a large dynamic range in response to one or more stimuli; 3) its expression profile exhibits a rapid kinetic response to one or more stimuli; 4) its expression profile is specific to a known biological pathway or a common set of

20   biological reactions or functions; 5) the regulon indicator gene does not contain sequences that are problematic for maintaining on plasmids when introduced into host cells. Most preferably, their expression is relatively specific for a particular biochemical pathway or cellular condition, highly sensitive to small changes in activation of a biochemical pathway or cellular condition and exhibit a wide dynamic

25   range of expression so that the RIG is easier to assay.

A "large dynamic range" is one in which the response in gene expression in response to a stimulus is at least four-fold over basal levels of expression in the absence of the stimulus. A response may be either an increase or a decrease in gene expression. In a preferred embodiment, the response is at least ten-fold over

30   basal levels. In a more preferred embodiment, the response is at least twenty-fold over

basal levels. In an even more preferred embodiment, the response is at least 100-fold over basal levels.

A "rapid kinetic response" is one in which the response occurs in the same time period as the doubling time of the organism after stimulation with the stimulus. In a preferred embodiment, the response occurs less than 10 minutes. In a more preferred embodiment, the response occurs in less than one minute.

A "stimulus" or "stimuli" is a chemical compound, a genetic mutation, or a change in the environment of the cell, including, without limitation, a change in pH, temperature, osmotic pressure, salinity, light, gas concentration or partial pressure (e.g. O_2, CO_2, CO or NO).

In order to determine whether a potential RIG is specific for a particular biochemical pathway or cellular condition, expression of the potential RIG is examined under all conditions in the expression database. A desirable RIG is one whose expression is selectively induced or repressed by chemicals or mutations that are known to affect the process in question. Likewise, a desirable RIG's expression is not influenced by chemicals or mutations that are known not to affect the process in question. This analysis provides information regarding whether the RIG participates in additional cellular processes or biochemical pathways. When a potential RIG is not a member of a target regulon, pathway or process, specificity is measured by analyzing expression under all conditions under which the potential RIG is activated or repressed to determine if similar conditions elicit similar responses.

Most preferably, a single RIG may be identified to be highly specific to a particular pathway, i.e., wherein its expression changes only when a particular pathway is activated or repressed, but not when other pathways are likewise regulated. Such a highly specific regulon indicator gene cannot always be found for a pathway of interest. In such cases, however, more than one RIG may be identified whose coordinate expression patterns correlate with high specificity to a pathway of interest. Preferably, the coordinate expression of two RIGs provides such specificity. However, the present invention is not limited by the number of RIGs identified and used simultaneously as regulated pathway indicators. Expression of each member of a

plurality of RIGs may independently increase or decrease when the biological pathway of interest is activated or repressed.

In order to determine whether a potential RIG is highly indicative of activation of a particular pathway, the gene will be activated or repressed to an expression level at least 2-fold higher or lower (if the gene is repressed) than when the pathway is not activated. In a preferred embodiment, the gene is activated or repressed to an expression level at least 10-fold higher or lower than the unactivated pathway. In a more preferred embodiment, the gene is activated or repressed to an expression level at least 20-fold higher or lower than the unactivated pathway. The expression level may be represented as a natural log ratio of treated/untreated expression values. See Figure 37, for example. In a preferred embodiment, the natural log ratio of a RIG is greater than 1, more preferably greater than 2.5, and even more preferably greater than 4.0 when the pathway or process is activated.

In order to determine the dynamic range of a potential RIG, the expression of the RIG is assessed by examining its expression in response to all the treatments and mutations in the database. In a preferred embodiment, there is a high level of change in RIG expression for small changes in activation of the pathway.

In one embodiment of the invention, expression of a regulon indicator gene correlates with the expression of at least one known gene in a group of coordinately expressed genes or provide a measure of the function of a biological process of interest. The RIG is identified by a method comprising the steps of:

a) comparing gene expression profiles of a plurality of genes in the database to generate expression correlation coefficients;

b) identifying based on their relative expression correlation coefficients a set of genes that are coordinately expressed;

c) selecting a set of genes from b) which comprises one or more genes known to function in a particular biological pathway, or a common set of biological reactions or functions;

d) selecting a member of the set of c) having one or more of the following characteristics:

1) its expression profile is sensitive to one or more stimuli;

2) its expression profile exhibits a large dynamic range in response to one or more stimuli;

3) its expression profile exhibits a rapid kinetic response to one or more stimuli;

4) its expression profile is specific to a known biological pathway or a common set of biological reactions or functions;

5) the regulon indicator gene does not contain sequences that are problematic for maintaining on plasmids when introduced into host cells.

The RIG may also be co-regulated with one or more genes in the group of coordinately expressed genes of c) above. In addition, the RIG may control the expression of at least one other gene in the group of coordinately expressed genes of c) above. The RIG may be a gene of previously unknown function.

In another embodiment, the invention provides a method for identifying a regulon indicator gene in a database of compiled gene expression profiles, wherein expression of the regulon indicator gene provides a measure of the function of a biological pathway or process of interest. The method comprises the steps of:

a) examining exemplary expression profiles in response to one or more chemical or genetic treatments which target the pathway or process of interest to generate reporter sensitivity data;

b) selecting a set of genes from a) which comprises one or more genes most significantly affected in response to the treatment or treatments; and

c) selecting at least one gene from b) whose expression profile is maximized for its specificity and sensitivity to the treatment or class of treatments in a) compared to its sensitivity to all other treatments in the database.

The regulon indicator gene may be co-regulated with one or more genes in the set of genes of a) or the regulon indicator gene, upon expression, controls the expression of at least one other gene in the in the set of genes of a).

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Methods to Identify Potential Target Genes and Targets

A regulon is identified as described above under "Methods to Identify Potential RIGs." In a preferred embodiment, a regulon will contain both characterized and uncharacterized genes. In many cases, the characterized genes will have a common function or will be part of the same biochemical pathway. For instance, a regulon of the isoprenoid pathway will contain characterized genes involved in sterol biosynthesis. Uncharacterized genes will then be analyzed in terms of whether they are likely to be part of the same biochemical pathway as the characterized genes. The sequence of uncharacterized genes will be compared to the sequence of genes of known function to determine if the uncharacterized genes or their gene products have any motifs common to characterized genes.

For instance, uncharacterized genes will be examined for domains indicating enzymatic functions, including, without limitation, kinase, protease and phosphorylase activities. Similarly, uncharacterized genes will be examined for domains indicating that they might be transcription factors, including, without limitation, zinc finger, PHD, steroid-binding and helix-loop-helix regions. Other domains of interest include lipid-binding and ATP-binding domains. Uncharacterized genes will also be examined for sequence similarities to secreted factors and receptors. In a preferred embodiment, target genes and their encoded target proteins are previously uncharacterized, highly correlated with a particular regulon containing genes for a specific pathway or process, and that appear to be an enzyme, secreted factor, receptor or transcription factor.

In a preferred embodiment, a novel regulon target gene may be selected from a database of compiled gene expression profiles. The target gene is selected comprising the steps of:

    a)   comparing gene expression profiles of a plurality of genes in the database to generate expression correlation coefficients;

    b)   identifying based on their expression correlation coefficients a set of genes that are coordinately expressed;

c)   selecting from b) a set of genes comprising one or more genes
of unknown function and one or more genes known to function
in a particular biological pathway, or a common set of biological
reactions or functions of interest;

5   d)   selecting from the set of c) at least one gene of unknown
function, Gene X, as a novel regulon target gene; wherein Gene
X is a gene whose expression profile closely correlates to the
expression profiles of the one or more genes of the set of c)
known to function in the particular biological pathway, or

10   common set of biological reactions or functions of interest.

The method may further comprise the step of generating individual
correlation coefficients between the gene expression profile of Gene X and a plurality
of genes in the database to assess the selectivity of Gene X as a novel regulon target
gene.   The method may further comprise the step of determining whether the protein

15   encoded by Gene X exhibits substantial homology to a human, non-human mammal,
avian, amphibian, fish, insect or plant protein, including, without limitation, the step of
hybridizing Gene X to genomic DNA from human, non-human mammal, avian,
amphibian, fish, insect or plant cells or tissue under low stringency conditions,
comparing the DNA sequence of Gene X to the DNA sequences from other organisms,

20   or obtaining an amino acid sequence encoded by Gene X and comparing it to amino
acid sequences from other organisms.   The DNA or amino acid sequences from other
organisms may be contained within a database and the DNA or amino acid sequence
encoded by Gene X may compared to the DNA or amino acid sequences from other
organisms using a computer algorithm such as blastp, tblastn or another algorithm that

25   utilizes string alignments.   The method for identifying a target may further comprise
the steps of:

a) disrupting the function of Gene X or its homolog in a yeast cell; and

b) identifying whether the function of Gene X is essential for yeast
germination, vegetative growth, pseudohyphal or hyphal growth.

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In another embodiment of the invention, genes that are regulated by regulon target genes of yeast or its mammalian homolog may be identified. The method comprises the steps of

5

    a)   overexpressing the target gene in host cells of a matrix comprising a plurality of units of cells, the cells in each unit containing a reporter gene operably linked to an expression control sequence derived from a gene of a selected organism; and

    b)   identifying genes that are either induced or repressed by overexpression of the target gene.

10

In a preferred embodiment, the target gene is selected from the group consisting of *YMR134w, YER034w, YJL105w, YKL077w, YGR046w, YJR041c, YER044c* and *YLR100w* and their mammalian homologs.

Methods for Constructing Mutant Yeast Strains

Once a potential target has been identified, one may disrupt the gene to
15 determine the effect of inhibiting the gene's activity has on the phenotype of the yeast cell. There are a number of methods well known in the art by which a person can disrupt a particular gene in yeast. One of skill in the art can disrupt an entire gene and create a null allele, in which no portion of the gene is expressed. One may also produce and express an allele comprising a portion of the gene which is not sufficient
20 for gene function. This may be done by inserting a nonsense codon into the sequence of the gene such that translation of the mutant mRNA transcript ends prematurely. One may also produce and express alleles containing point mutations, individually or in combination, that reduce or abolish gene function.

There are a number of different strategies for creating conditional
25 alleles of genes. Broadly, an allele can be conditional for function or expression. An example of an allele that is <u>conditional for function</u> is a temperature sensitive mutation where the gene product is functional at one temperature but non-functional at another, e.g., due to misfolding or mislocalization. One of ordinary skill in the art may produce mutant alleles which may have only one or a few altered nucleotides but which encode

inactive or temperature-sensitive proteins. Temperature-sensitive mutant yeast strains express a functional protein at permissive temperatures but do not express a functional protein at non-permissive temperatures.

An example of an allele that is <u>conditional for expression</u> is a chimeric gene where a regulated promoter controls the expression of the gene. Under one condition the gene is expressed and under another it is not. One may replace or alter the endogenous promoter of the gene with a heterologous or altered promoter that can be activated only under certain conditions. These conditional mutants only express the gene under defined experimental conditions. In a preferred embodiment, the gene is under the control of a regulated promoter where the gene may be expressed at higher or lower levels depending upon the degree of activation of the promoter. For instance, a gene under the control of a regulated promoter may be expressed at any level between 0 and 100% of wild type expression, such as at 10%, 20%, 50% or 80% of its wild type level. The gene may also be expressed at levels above its usual wild type expression (overexpression). All of these methods are well known in the art. For example, see Stark (1998), Garfinkel et al., (1998), and Lawrence and Rothstein, (1991), herein incorporated by reference.

One having ordinary skill in the art also may decrease expression of a gene without disrupting or mutating the gene. For instance, one may decrease the expression of a gene by transforming yeast with an antisense molecule or ribozyme under the control of a regulated or constitutive promoter (see Nasr et al., 1995, herein incorporated by reference). One may introduce an antisense construct operably linked to an inducible promoter into *S. cerevisiae* to study the function of a conditional allele (see Nasr et al. supra). One problem that may be encountered, however, is that many antisense molecules do not work well in yeast, for reasons that are, as yet, unclear (see Atkins et al., 1994 and Olsson et al., 1997).

One may also decrease gene expression by inserting a sequence by homologous recombination into or next to the gene of interest wherein the sequence targets the mRNA or the protein for degradation. For instance, one can introduce a construct that encodes ubiquitin such that a ubiquitin fusion protein is produced. This

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protein will be likely to have a shorter half-life than the wildtype protein. See, e.g., Johnson et al. (1992), herein incorporated by reference.

In a preferred mode, a gene of interest is completely disrupted in order to ensure that there is no residual function of the gene. One can disrupt a gene by

5   "classical" or PCR-based methods. The "classical" method of gene knockout is described by Rothstein (1991), herein incorporated by reference. However, it is preferable to use a PCR-based deletion method because it is faster and less labor intensive.

A preferred method to delete a gene is a one-step, polymerase chain

10   reaction (PCR) based gene deletion method (Rothstein, 1991). Gene specific primer pairs are designed for PCR amplification of the plasmid pFA6a-KanMX4 (Wach et al., 1994), which teachings are herein incorporated by reference. The 3' ends of the upstream and downstream gene specific primers have been designed to include 18 basepairs (bp) and 19 bp, respectively, of nucleotide homology flanking the KanMX

15   gene of the plasmid pFA6a-KanMX4 template. All of the gene specific primer pairs contain these complementary sequences, such that the same plasmid pFA6a-KanMX4 template can be used for all of the first round PCR reactions. At their 5' ends, the primers each have gene specific sequence homologies. The upstream primer contains a nucleotide sequence which includes the start codon of the gene to be knocked out and

20   the sequence immediately upstream of the start codon. The downstream primer contains a nucleotide sequence which includes the stop codon of the gene and the sequence immediately downstream of the stop codon. For each set of primers, the sequences of the gene are derived from the 5' and 3' ends of the target DNA sequence.

The upstream and downstream primers are then used to amplify the

25   pFA6a-KanMX4 by PCR using standard conditions for PCR. Hybridization conditions for specific gene-specific primers can be experimentally determined, or estimated by a number of formulas. One such formula is $T_m = 81.5 + 16.6 \, (\log_{10}[Na^+]) + 0.41$ (fraction G + C) - (600/N). See Sambrook et al. pages 11.46-11.47. The products of the first round PCR reactions are DNA molecules containing the KanMX marker

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(conferring resistance to the drug G-418 in *S. cerevisiae*) flanked on both ends by 18 bp of gene specific sequences.

The gene specific flanking sequences are extended during the second round PCR reactions. The sequences of the two gene specific PCR primers are

5   derived from the 45 bp immediately upstream (including the start codon) and the 45 bp immediately downstream (including the stop codon) of each gene. Thus, following the second round of PCR the product contains the KanMX marker flanked by 45 bp of gene specific sequences corresponding to the sequences flanking the gene's ORF. The PCR products are purified by an isopropanol precipitation, and shipped with the

10   analytical primers (see below) to the consortium members on dry ice. The precipitated PCR products are resuspended in TE buffer (10 mM Tris-HCl [pH 7.6], 1 mM EDTA).

The various mutations are constructed in two related *Saccharomyces cerevisiae* strains, BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and BY4743

15   (*MATa/MATα his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 LYS2/lys2Δ0 met15Δ0/MET15 ura3Δ0/ura3Δ0*) (Brachmann et al., 1998). Both of these strains are transformed with the PCR products by the lithium acetate method as described by Ito et al., 1983, and Schiestl and Gietz, 1989, herein incorporated by reference. The flanking, gene-specific yeast sequences target the integration event by homologous recombination to

20   the desired locus (Figure 1). Transformants are selected on rich medium (YPD) which contains G-418 (Geneticin, Life Technologies, Inc.) as described by Guthrie and Fink, 1991, herein incorporated by reference. Ideally, independent mutations are isolated in the haploid (BY4741) and the diploid (BY4743) strains. The heterozygous mutant diploid strain is then sporulated, and subjected to tetrad analysis (Sherman, 1991;

25   Sherman and Wakem, 1991, herein incorporated by reference). This allows for the isolation of the mutation in a *MATα* haploid strain. The two independently isolated *MATa* and *MATα* haploid strains are then mated to create a homozygous mutant diploid strain.

Methods to Characterize Yeast Gene Function

One of skill in the art will recognize that a number of methods can be used to characterize the function of a yeast gene. In general, the preferred strategy depends upon the assumptions made regarding the function of the gene. For example, if one creates a conditional allele of the gene, then one can engineer a mutant strain wherein the wildtype allele has been replaced by a conditional allele. See, e.g., Stark (1998). The strain is constructed and propagated under the permissive condition, and then the strain is switched to the non-permissive (or restrictive) condition and effects upon the cell's phenotype is monitored. This can be done in a haploid cell, or in a diploid cell as either a homozygous or heterozygous mutant. .

A preferred method of characterizing the function of a gene is to knockout the gene completely and then analyze the knockout yeast strain by tetrad analysis. This method is preferred because one does not need to be able to engineer a conditional allele. Furthermore, as the knockout is a null allele, one is assured that it is the null phenotype that is assessed, rather than a phenotype resulting from a potentially hypomorphic conditional allele. In addition, a complete knockout of the gene can be constructed in a diploid strain where the potentially essential function of the gene is complemented by the second copy of the gene.

Once the knockout has been constructed as a heterozygous mutant, the effects of the mutation is assessed in the haploid spores. Tetrad analysis of the haploid spores allows for the genetic characterization of a mutation because one can determine the effect of the homozygous gene linked to the knockout marker (G-418 resistance).

Any of a number of different tests can be performed to determine the effect of knocking out the selected target gene. For instance, one can determine whether the yeast cell is more or less responsive to various pharmaceutical compounds (e.g., see Figure 4), pH, salinity, osmotic pressure, temperature or nutritional conditions. One can determine whether the knockout results in a different observable phenotype (e.g., see Figure 22). In addition, yeast cells can be tested for their ability to mate, sporulate and bud relative to a wild type control. Thus, these tests may provide important information regarding the function of the target gene.

Methods to Identify Potential Homologs in Other Organisms

Once a gene has been identified as a potential target, one can determine whether the gene from yeast has homologs in other organisms, such as humans, non-human mammals, other vertebrates such as fish, insects, plants, or other fungi.

5   One method of determining whether an *S. cerevisiae* gene has homologs is by the use of low stringency hybridization and washing. In general, genomic DNA or cDNA libraries can be screened using probes derived from the target *S. cerevisiae* gene using methods known in the art. See above and pages 8.46-8.49 and 9.46-9.58 of Sambrook et al., 1989, herein incorporated by reference. Preferably,

10   genomic DNA libraries are screened because cDNA libraries generally will not contain all the mRNA species an organism can make. Genomic DNA libraries from a variety of different organisms, such as plants, fungi, insects, and various mammalian species are commercially available and can be screened. This method is useful for determining whether there are homologs in organisms whose DNA sequences have not been

15   characterized extensively.

A second method of determining whether an *S. cerevisiae* gene has homologs is through the use of degenerate PCR. In this method, degenerate oligonucleotides that encode short amino acid sequences of the *S. cerevisiae* gene are made. Methods of preparing degenerate oligonucleotides and using them in PCR to

20   isolate uncloned genes are well known in the art (see Sambrook, pages 14.7-14.8, and Crawley et al., 1997, pages 4.2.1-4.2.5, herein incorporated by reference).

The most preferred method is to compare the sequence of the *S. cerevisiae* gene to sequences from other organism. Either the nucleotide sequence of the gene or its encoded amino acid sequence is compared to the sequences from other

25   organisms. Preferably, the encoded amino acid sequence of the yeast gene is compared to amino acid sequences from other organisms. The sequence of the yeast gene can be compared by a number of different algorithms well known in the art. In general, computer programs designed for sequence analysis are used for the purpose of comparing the sequence of interest to a large database of other sequences. Any

30   computer program designed for the purpose of sequence comparison can be used in

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this method. Some computer programs, such as Fasta, produce results that are
typically presented as "% sequence identity." Other computer programs, such as
blastp, produce results presented as "p-values." Preferably, the target gene sequence
will be compared to other sequences using the blastp algorithm.

5   Nucleotide and amino acid sequences of target genes may be compared
to vertebrate sequences, including human and non-human mammalian sequences, as
well as plant and insect sequences using any one of the large number of programs
known in the art for comparing nucleotide and amino acid sequences to sequences in a
database. Examples of such programs are Fasta and blastp, discussed above.

10   Examples of databases which can be searched include GenBank-EMBL, SwissProt,
DDBJ, GeneSeq, and EST databases, as well as databases containing combinations of
these databases.

          As a further characterization, any potential homologs from other
organisms can be assessed for their ability to functionally complement the yeast

15   mutant. This can be achieved by first cloning the homolog into a *S. cerevisiae*
expression vector by standard methods. This plasmid can then be transformed into the
heterozygous mutant diploid strain. Upon sporulation and tetrad dissection the ability
of the homolog to complement the yeast function is determined by whether or not the
haploid spores complements the yeast knockout and restores the wildtype function of

20   the haploid spore. The ability of the homolog to complement the yeast mutant would
indicate shared function(s) and suggest that the homolog may be part of a similar
pathway in the other organism.

Nucleic Acids, Vectors and Production of Recombinant Polypeptides

          The present invention provides nucleic acids and recombinant DNA

25   vectors which comprise *S. cerevisiae* RIG and target gene DNA sequences.
Specifically, vectors comprising all or portions of the DNA sequence of *HES1*,
YMR134w, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and
YLR100w are provided. The vectors of this invention also include those comprising
DNA sequences which hybridize under stringent conditions to the *HES1*, *YMR134w*,

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YER034w, YJL105w, YKL077w, YGR046w, YJR041c, YER044c and *YLR100w* gene
sequences, and conservatively modified variations thereof.

     The nucleic acids of this invention include single-stranded and double-
stranded DNA, RNA, oligonucleotides, antisense molecules, or hybrids thereof and

5   may be isolated from biological sources or synthesized chemically or by recombinant
DNA methodology. The nucleic acids, recombinant DNA molecules and vectors of
this invention may be present in transformed or transfected cells, cell lysates, or in
partially purified or substantially pure forms.

     DNA sequences may be expressed by operatively linking them to an

10   expression control sequence in an appropriate expression vector and employing that
expression vector to transform an appropriate unicellular host. Expression control
sequences are sequences which control the transcription, post-transcriptional events
and translation of DNA sequences. Such operative linking of a DNA sequence of this
invention to an expression control sequence, of course, includes, if not already part of

15   the DNA sequence, the provision of a translation initiation codon, ATG, in the correct
reading frame upstream of the DNA sequence.

     A wide variety of host/expression vector combinations may be
employed in expressing the DNA sequences of this invention. Useful expression
vectors, for example, may consist of segments of chromosomal, non-chromosomal and

20   synthetic DNA sequences.

     Useful expression vectors for bacterial hosts include bacterial plasmids,
such as those from *E. coli*, including pBluescript, pGEX-2T, pUC vectors, col E1,
pCR1, pBR322, pMB9 and their derivatives, wider host range plasmids, such as RP4,
phage DNAs, e.g., the numerous derivatives of phage lambda, e.g., NM989, λGT10

25   and λGT11, and other phages, e.g., M13 and filamentous single stranded phage DNA.
In yeast, vectors include Yeast Integrating plasmids (*e.g.*, YIp5) and Yeast Replicating
plasmids (the YRp and YEp series plasmids), Yeast centromere plasmids (the YCp
series plasmids), pGPD-2, 2μ plasmids and derivatives thereof, and improved shuttle
vectors such as those described in Gietz and Sugino, Gene, 74, pp. 527-34 (1988)

30   (YIplac, YEplac and YCplac). Expression in mammalian cells can be achieved using a

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variety of plasmids, including pSV2, pBC12BI, and p91023, as well as lytic virus vectors (*e.g.*, vaccinia virus, adeno virus, and baculovirus), episomal virus vectors (*e.g.*, bovine papillomavirus), and retroviral vectors (*e.g.*, murine retroviruses). Useful vectors for insect cells include baculoviral vectors and pVL 941.

5   In addition, any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence when operatively linked to it -- may be used in these vectors to express the DNA sequences of this invention. Such useful expression control sequences include the expression control sequences associated with structural genes of the foregoing expression vectors. Expression

10   control sequences that control transcription include, e.g., promoters, enhancers and transcription termination sites. Expression control sequences that control post-transcriptional events include splice donor and acceptor sites and sequences that modify the half-life of the transcribed RNA, e.g., sequences that direct poly(A) addition or binding sites for RNA-binding proteins. Expression control sequences that

15   control translation include ribosome binding sites, sequences which direct expression of the polypeptide to particular cellular compartments, and sequences in the 5' and 3' untranslated regions that modify the rate or efficiency of translation.

Examples of useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the lac system, the trp system, the

20   TAC or TRC system, the T3 and T7 promoters, the major operator and promoter regions of phage lambda, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast α-mating system, the GAL1 or GAL10 promoters, and other constitutive and inducible promoter sequences known to

25   control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. See, e.g., The Molecular Biology of the Yeast Saccharomyces (eds. Strathern, Jones and Broach) Cold Spring Harbor Lab., Cold Spring Harbor, N.Y. for details on yeast molecular biology in general and on yeast expression systems (pp. 181-209) (incorporated herein by reference)).

DNA vector design for transfection into mammalian cells should include appropriate sequences to promote expression of the gene of interest, including: appropriate transcription initiation, termination and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. A great number of expression control sequences -- constitutive, inducible and/or tissue-specific -- are known in the art and may be utilized. For eukaryotic cells, expression control sequences typically include a promoter, an enhancer derived from immunoglobulin genes, SV40, cytomegalovirus, etc., and a polyadenylation sequence which may include splice donor and acceptor sites. Substantial progress in the development of mammalian cell expression systems has been made in the last decade and many aspects of the system are well characterized.

Preferred DNA vectors also include a marker gene and means for amplifying the copy number of the gene of interest. DNA vectors may also comprise stabilizing sequences (e.g., ori- or ARS-like sequences and telomere-like sequences), or may alternatively be designed to favor directed or non-directed integration into the host cell genome. In a preferred embodiment, DNA sequences of this invention are inserted in frame into an expression vector that allows high level expression of an RNA which encodes a fusion protein comprising encoded DNA sequence of interest.

Of course, not all vectors and expression control sequences will function equally well to express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. However, one of skill in the art may make a selection among these vectors, expression control sequences and hosts without undue experimentation and without departing from the scope of this invention. For example, in selecting a vector, the host must be considered because the vector must be replicated in it. The vector's copy number, the ability to control that copy number, the ability to control integration, if any, and the expression of any other

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proteins encoded by the vector, such as antibiotic or other selection markers, should also be considered.

In selecting an expression control sequence, a variety of factors should also be considered. These include, for example, the relative strength of the sequence,

5   its controllability, and its compatibility with the DNA sequence of this invention, particularly with regard to potential secondary structures. Unicellular hosts should be selected by consideration of their compatibility with the chosen vector, the toxicity of the product coded for by the DNA sequences of this invention, their secretion characteristics, their ability to fold the polypeptide correctly, their fermentation or

10   culture requirements, and the ease of purification from them of the products coded for by the DNA sequences of this invention.

Within these parameters, one of skill in the art may select various vector/expression control sequence/host combinations that will express the DNA sequences of this invention in fermentation or in other large scale cultures.

15   Given the strategies described herein, one of skill in the art can construct a variety of vectors and nucleic acid molecules comprising functionally equivalent nucleic acids. DNA cloning and sequencing methods are well known to those of skill in the art and are described in an assortment of laboratory manuals, including Sambrook et al, supra, 1989; and Ausubel et al., 1994 Supplement. Product

20   information from manufacturers of biological, chemical and immunological reagents also provide useful information.

The recombinant DNA molecules and more particularly, the expression vectors of this invention may be used to express the RIG and target genes from *S. cerevisiae* as recombinant polypeptides in a heterologous host cell. The polypeptides

25   of this invention may be full-length or less than full-length polypeptide fragments recombinantly expressed from the DNA sequences according to this invention. Such polypeptides include variants and muteins having biological activity. The polypeptides of this invention may be soluble, or may be engineered to be membrane- or substrate-bound using techniques well known in the art.

Particular details of the transfection, expression and purification of recombinant proteins are well documented and are understood by those of skill in the art. Further details on the various technical aspects of each of the steps used in recombinant production of foreign genes in mammalian cell expression systems can be

5   found in a number of texts and laboratory manuals in the art. See, e.g., Ausubel et al., 1989, herein incorporated by reference.

Transformation and other methods of introducing nucleic acids into a host cell (e.g., transfection, electroporation, liposome delivery, membrane fusion techniques, high velocity DNA-coated pellets, viral infection and protoplast fusion) can

10   be accomplished by a variety of methods which are well known in the art (see, for instance, Ausubel, *supra,* and Sambrook, *supra*). Bacterial, yeast, plant or mammalian cells are transformed or transfected with an expression vector, such as a plasmid, a cosmid, or the like, wherein the expression vector comprises the DNA of interest. Alternatively, the cells may be infected by a viral expression vector comprising the

15   DNA or RNA of interest. Depending upon the host cell, vector, and method of transformation used, transient or stable expression of the polypeptide will be constitutive or inducible. One having ordinary skill in the art will be able to decide whether to express a polypeptide transiently or stably, and whether to express the protein constitutively or inducibly.

20   A wide variety of unicellular host cells are useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli, Pseudomonas, Bacillus, Streptomyces,* fungi, yeast, insect cells such as *Spodoptera frugiperda* (SF9), animal cells such as CHO, BHK, MDCK and various murine cells, e.g., 3T3 and WEHI cells, African green

25   monkey cells such as COS 1, COS 7, BSC 1, BSC 40, and BMT 10, and human cells such as VERO, WI38, and HeLa cells, as well as plant cells in tissue culture.

Expression of recombinant DNA molecules according to this invention may involve post-translational modification of a resultant polypeptide by the host cell. For example, in mammalian cells expression might include, among other things,

30   glycosylation, lipidation or phosphorylation of a polypeptide, or cleavage of a signal

sequence to produce a "mature" protein. Accordingly, the polypeptide expression products of this invention encompass full-length polypeptides and modifications or derivatives thereof, such as glycosylated versions of such polypeptides, mature proteins and polypeptides retaining a signal peptide. The present invention also provides for

5   biologically active fragments of the polypeptides. Sequence analysis or genetic manipulation may identify those domains responsible for the function of the protein in yeast. Thus, the invention encompasses the production of biologically active fragments. The invention also encompasses fragments of the polypeptides which would be valuable as antigens for the production of antibodies, or as competitors for

10   antibody binding.

The polypeptides of this invention may be fused to other molecules, such as genetic, enzymatic or chemical or immunological markers such as epitope tags. Fusion partners include, *inter alia*, *myc*, hemagglutinin (HA), GST, immunoglobulins, β-galactosidase, biotin trpE, protein A, β-lactamase, α amylase, maltose binding

15   protein, alcohol dehydrogenase, polyhistidine (for example, six histidine at the amino and/or carboxyl terminus of the polypeptide), lacZ, green fluorescent protein (GFP), yeast α mating factor, GAL4 transcription activation or DNA binding domain, luciferase, and serum proteins such as ovalbumin, albumin and the constant domain of IgG. See, e.g., Godowski et al., 1988, and Ausubel et al., *supra*. Fusion proteins may

20   also contain sites for specific enzymatic cleavage, such as a site that is recognized by enzymes such as Factor XIII, trypsin, pepsin, or any other enzyme known in the art. Fusion proteins will typically be made by either recombinant nucleic acid methods, as described above, chemically synthesized using techniques such as those described in Merrifield, 1963, herein incorporated by reference, or produced by chemical cross-

25   linking.

Tagged fusion proteins permit easy localization, screening and specific binding via the epitope or enzyme tag. See Ausubel, 1991, Chapter 16. Some tags allow the protein of interest to be displayed on the surface of a phagemid, such as M13, which is useful for panning agents that may bind to the desired protein targets.

Thus, fusion proteins are useful for screening potential agents using the proteins encoded by the target genes.

One advantage of fusion proteins is that an epitope or enzyme tag can simplify purification. These fusion proteins may be purified, often in a single step, by affinity chromatography. For example, a His[6] tagged protein can be purified on a Ni affinity column and a GST fusion protein can be purified on a glutathione affinity column. Similarly, a fusion protein comprising the Fc domain of IgG can be purified on a Protein A or Protein G column and a fusion protein comprising an epitope tag such as myc can be purified using an immunoaffinity column containing an anti-c-myc antibody. It is preferable that the epitope tag be separated from the protein encoded by the target gene by an enzymatic cleavage site that can be cleaved after purification. A second advantage of fusion proteins is that the epitope tag can be used to bind the fusion protein to a plate or column through an affinity linkage for screening targets.

In addition, fusion proteins comprising the constant domain of IgG or other serum proteins can increase a protein's half-life in circulation for use therapeutically. Fusion proteins comprising a targeting domain can be used to direct the protein to a particular cellular compartment or tissue target in order to increase the efficacy of the functional domain. See, e.g., U.S. Pat. No. 5,668,255, which discloses a fusion protein containing a domain which binds to an animal cell coupled to a translocation domain of a toxin protein. Fusion proteins may also be useful for improving antigenicity of a protein target. Examples of making and using fusion proteins are found in U.S. Pat. Nos. 5,225,538, 5,821,047, and 5,783,398, which are hereby incorporated by reference.

Production of Polypeptide Fragments, Derivatives and Muteins and Biological Assays Thereof

Fragments, derivatives and muteins of polypeptides encoded by the RIG and target genes can be produced recombinantly or chemically, as discussed above. One can produce fragments of a polypeptide encoding a target gene by truncating the DNA encoding the target gene and then expressing it recombinantly. Alternatively,

one can produce a fragment by chemically synthesizing a portion of the full-length polypeptide. One may also produce a fragment by enzymatically cleaving the polypeptide. Methods of producing polypeptide fragments are well-known in the art (see, e.g., Sambrook et al. and Ausubel et al. *supra*).

One may produce muteins of a polypeptide encoded by a target gene by introducing mutations into the DNA sequence of the gene and then expressing it recombinantly. These mutations may be targeted, in which particular encoded amino acids are altered, or may be untargeted, in which random encoded amino acids within the polypeptide are altered. Muteins with random amino acid alterations can be screened for a particular biological activity. Methods of producing muteins with targeted or random amino acid alterations are well known in the art, see e.g., Sambrook et al., Ausubel et al., *supra,* and U.S. Pat. No. 5,223,408, herein incorporated by reference. Production of polypeptide derivatives are well known in the art, see above.

There are a number of methods known in the art to determine whether fragments, muteins and derivatives of polypeptides encoded by a target gene has the same, enhanced or decreased biological activity as the wild type polypeptide. One of the simplest assays involves determining whether the fragment, mutein or derivative can complement the gene function in a cell which does not contain the target gene. For instance, one can introduce a DNA encoding a fragment or mutein of a polypeptide encoded by a gene into a mutant yeast strain which has the gene of interest deleted (see above under "Methods of Producing Mutant Yeast Strains"). If introduction of the DNA encoding the fragment or mutein permits the mutant yeast strain to regain its wildtype phenotype, then the fragment or mutein is biologically active, and complements the deleted gene.

In one type of screening assay, the target gene or a fragment thereof can be used as the "bait" in a two-hybrid screen to identify molecules that physically interact with the target gene. See Chien et al. (1991).

In addition, one may generate genome expression profiles of yeast strains to characterize the gene's function. In order to generate such profiles, a non-

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functional or conditional allele of the gene in a yeast strain must be produced. The conditional or non-functional allele may be constructed by any technique known in the art, including deleting the gene as described above, making a temperature-sensitive allele of the gene or operably linking the gene to an inducible promoter for regulated expression. If the yeast strain contains a non-functional allele, a genome expression profile of the mutant strain is compared to a wild type strain. If the yeast strain contains a conditional allele, the yeast strain is first grown under the permissive condition to permit expression of the functional product of the targetl gene. Then, the yeast strain is shifted to the nonpermissive condition, in which the product of the target gene is not made or is non-functional. The genome expression profile of the yeast strain under the nonpermissive condition may be compared to the same yeast strain grown under permissive conditions or a wildtype yeast strain. Structure-function studies can be performed wherein a library of mutant forms of the gene is screened for the ability to complement the knock-out mutant strain.

Fragments, muteins and derivatives may also be micro-injected into a mutant yeast strain in which the gene of interest is deleted to determine whether the introduction of the fragment, mutein or derivative can complement the genetic defect. Similarly, fragments, muteins and derivatives may be microinjected into other cell types in which the homologous gene has been deleted.

Finally, if a particular biochemical activity of a polypeptide encoded by a target gene is known, this activity can be measured for fragments, muteins or derivatives of the polypeptide. For instance, if a target gene encodes a kinase, one could measure the kinase activity of the wild type polypeptide and compare it to the activity of a fragment, mutein or derivative.

Production of Antibodies

The polypeptides encoded by the target genes of this invention may be used to elicit polyclonal or monoclonal antibodies which bind to the target gene product or a homolog from another species using a variety of techniques well known to those of skill in the art. Alternatively, peptides corresponding to specific regions of

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the polypeptide encoded by the target gene may be synthesized and used to create immunological reagents according to well known methods.

Antibodies directed against the polypeptides of this invention are immunoglobulin molecules or portions thereof that are immunologically reactive with
5   the polypeptide of the present invention. It should be understood that the antibodies of this invention include antibodies immunologically reactive with fusion proteins.

Antibodies directed against a polypeptide encoded by a target gene may be generated by immunization of a mammalian host. Such antibodies may be polyclonal or monoclonal. Preferably they are monoclonal. Methods to produce
10   polyclonal and monoclonal antibodies are well known to those of skill in the art. For a review of such methods, see Harlow and Lane (1988), Yelton et al. (1981), and Ausubel et al. (1989) herein incorporated by reference. Determination of immunoreactivity with a polypeptide encoded by an target gene may be made by any of several methods well known in the art, including by immunoblot assay and ELISA.

15   Monoclonal antibodies with affinities of 10^{-8} M^{-1} or preferably 10^{-9} to 10^{-10} M^{-1} or stronger are typically made by standard procedures as described, e.g., in Harlow and Lane, 1988 or Goding, 1986. Briefly, appropriate animals are selected and the desired immunization protocol followed. After the appropriate period of time, the spleens of such animals are excised and individual spleen cells fused, typically, to
20   immortalized myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone tested for their production of an appropriate antibody specific for the desired region of the antigen.

Other suitable techniques involve *in vitro* exposure of lymphocytes to the antigenic polypeptides, or alternatively, to selection of libraries of antibodies in
25   phage or similar vectors. See Huse et al., 1989. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the
30   scientific and patent literature. Suitable labels include radionuclides, enzymes,

substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic

particles and the like. Patents teaching the use of such labels include U.S. Patents

3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241,

herein incorporated by reference. Also, recombinant immunoglobulins may be

5   produced (see U.S. Patent 4,816,567, herein incorporated by reference).

An antibody of this invention may also be a hybrid molecule formed

from immunoglobulin sequences from different species (e.g., mouse and human) or

from portions of immunoglobulin light and heavy chain sequences from the same

species. An antibody may be a single-chain antibody or a humanized antibody. It may

10   be a molecule that has multiple binding specificities, such as a bifunctional antibody

prepared by any one of a number of techniques known to those of skill in the art

including the production of hybrid hybridomas, disulfide exchange, chemical cross-

linking, addition of peptide linkers between two monoclonal antibodies, the

introduction of two sets of immunoglobulin heavy and light chains into a particular cell

15   line, and so forth.

The antibodies of this invention may also be human monoclonal

antibodies, for example those produced by immortalized human cells, by SCID-hu mice

or other non-human animals capable of producing "human" antibodies, or by the

expression of cloned human immunoglobulin genes. The preparation of humanized

20   antibodies is taught by U.S. Pat. Nos. 5,777,085 and 5,789,554, herein incorporated by

reference.

In sum, one of skill in the art, provided with the teachings of this

invention, has available a variety of methods which may be used to alter the biological

properties of the antibodies of this invention including methods which would increase

25   or decrease the stability or half-life, immunogenicity, toxicity, affinity or yield of a

given antibody molecule, or to alter it in any other way that may render it more suitable

for a particular application.

Therapeutic Methods Using Nucleic Acids Encoding Target Genes

Once a target gene has been identified in *S. cerevisiae*, the gene and its nucleotide sequence can be exploited in a number of ways depending upon the nature of the target gene. One method is to use the primary sequence of the target gene itself.

5   For instance, antisense oligonucleotides can be produced which are complementary to the mRNA of the target gene. Antisense oligonucleotides can be used to inhibit transcription or translation of a target yeast gene. Production of antisense oligonucleotides effective for therapeutic use is well-known in the art, see Agrawal et al., 1998, Lavrovsky et al., 1997, and Crooke, 1998, herein incorporated by reference.

10 Antisense oligonucleotides are often produced using derivatized or modified nucleotides in order to increase half-life or bioavailability.

The primary sequence of the target gene can also be used to design ribozymes that can target and cleave specific target gene sequences. There are a number of different types of ribozymes. Most synthetic ribozymes are generally

15 hammerhead, *Tetrahymena* and hairpin ribozymes. Methods of designing and using ribozymes to cleave specific RNA species are known in the art, see Zhao et al., 1998, Larovsky et al., 1997, and Eckstein, 1997, herein incorporated by reference. Although hammerhead ribozymes are generally ineffective in yeast (Castanotto et al., 1998), other types of ribozymes may be effective in yeast, and hammerhead and other types of

20 ribozymes are effective in other organisms.

As discussed above, one can use target yeast genes to identify homologous genes in plants and animals, including humans. Therefore, one can design ribozymes and antisense molecules to these genes from plants and animals, including humans.

25 **Methods Using Neutralizing Antibodies to Proteins Encoded by Target Genes**

The protein encoded by the target gene can be used to elicit neutralizing antibodies for use as inhibit the function of the target protein. An antibody may be an especially good inhibitor if the target gene of interest encodes a protein which is expressed on the cell surface, such as an integral membrane protein. Although

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polyclonal antibodies may be made, monoclonal antibodies are preferred. Monoclonal antibodies can be screened individually in order to isolate those that are neutralizing or inhibitory for the protein encoded by the target gene. Monoclonal antibodies also may be screened for inhibition of a particular function of a protein. For instance, if it is

5   known that the target gene in yeast encodes an enzyme, one can identify antibodies that inhibit the enzymatic activity. Alternatively, if the specific function of a target gene is unknown, one can measure inhibition of the protein by determining the genome expression profile for yeast cells contacted with the neutralizing antibody. Similarly, one can screen antibodies which are directed against animal, plant or human proteins

10   for inhibition of the protein's activity in appropriate cells.   .

Monoclonal antibodies which inhibit a target protein *in vitro* may be humanized for therapeutic use using methods well-known in the art, see, e.g., U.S. Pat. Nos. 5,777,085 and 5,789,554, herein incorporated by reference. Monoclonal antibodies may also be engineered as single-chain antibodies using methods well-

15   known in the art for therapeutic use, see, e.g., U.S. Pat. Nos. 5,091,513, 5,587,418, and 5,608,039, herein incorporated by reference.

Neutralizing antibodies may also be used diagnostically. For instance, the binding site of a neutralizing antibody to the protein encoded by the target gene can be used to help identify domains that are required for the protein's activity. The

20   information about the critical domains of a target protein can be used to design inhibitors that bind to the critical domains of the target protein. In addition, neutralizing antibodies can be used to validate whether a potential inhibitor of an target protein inhibits the protein in *in vitro* assays.

Methods of Identifying Functional Attributes of the Target

25   Once a target gene in yeast is identified, the GRM (or an equivalent) is used to help identify critical functional attributes of the gene. In order to determine the particular transcripts a target gene modifies, one overexpresses the target gene in the cells of the GRM. One may also overexpress a conditional allele of the gene in the cells of the GRM. Then, one identifies a subset of genes that are either induced or

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repressed by overexpression of the target gene. Methods for processing data using the GRM are also disclosed in United States Patents 5,569,588 and 5,777,888; see also United States Patent Application Serial No. 09/076,668, now pending. Once the genes that are regulated by a target gene are identified, one can use this information in a

5   number of ways to identify potential inhibitors or activators of the target protein. Alternatively, one may determine the genome expression profile of a cell that has a mutation in a target gene, or a cell that has the endogenous target gene replaced either with an altered allele or with the counterpart gene from another species. Similarly, plant and animal GRMs, including human GRMs, overexpressing target genes can be

10   used in the same way to identify potential inhibitors or activators of the target protein in these organisms.

Another method for isolating a potential inhibitors or activators of a target gene is to use information obtained from the "two-hybrid system" to identify and clone genes encoding proteins that interact with the polypeptide encoded by the target

15   gene (see, e.g., Chien et al.,1991, incorporated herein by reference). The amino acid sequences of the polypeptides identified by the two-hybrid system can be used to design inhibitory peptides to the target protein. The "two-hybrid" system using libraries of the appropriate species can also be used to identify and clone genes encoding proteins that interact with the polypeptide encoded by the target genes.

20   **Methods of Using Target Proteins**

Recombinantly expressed target proteins or functional fragments thereof can be used to screen libraries of natural, semisynthetic or synthetic compounds. Particularly useful types of libraries include combinatorial small organic molecule libraries, phage display libraries, and combinatorial peptide libraries.

25   Methods of determining whether components of the library bind to a particular polypeptide are well known in the art. In general, the polypeptide target is attached to solid support surface by non-specific or specific binding. Specific binding can be accomplished using an antibody which recognizes the protein that is bound to a solid support, such as a plate or column. Alternatively, specific binding may be through an

epitope tag, such as GST binding to a glutathione-coated solid support, or IgG fusion protein binding to a Protein A solid support. Alternatively, the recombinantly expressed protein or fragments thereof may be expressed on the surface of phage, such as M13. A library in mobile phase is incubated under conditions to promote specific binding between the target and a compound. Compounds which bind to the target can then be identified. Alternately, the library is attached to a solid support and the polypeptide target is in the mobile phase.

Binding between a compound and target can be determined by a number of methods. The binding can be identified by such techniques as competitive ELISAs or RIAs, for example, wherein the binding of a compound to a target will prevent an antibody to the target from binding. These methods are well-known in the art, see, e.g., Harlow and Lane, *supra*. Another method is to use BiaCORE (BiaCORE) to measure interactions between a target and a compound using methods provided by the manufacturer. A preferred method is automated high throughput screening, see, e.g., Burbaum et al., 1997, and Schullek et al., 1997, herein incorporated by reference.

Once a compound that binds to a target is identified, one then determines whether the compound inhibits the activity of the target. If a biological function for the target protein is known, one could determine whether the compound inhibited the biological activity of the protein. For instance, if it is known that the target protein is an enzyme, one can measure the inhibition of enzymatic activity in the presence of the potential inhibitor.

In a preferred embodiment, the target gene is selected from *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

Another embodiment of the invention is to use the recombinantly expressed protein for rational drug design. The structure of the recombinant protein may be determined using x-ray crystallography or nuclear magnetic resonance (NMR). Alternatively, one could use computer modeling to determine the structure of the protein. The structure can be used in rational drug design to design potential inhibitory

compounds of the target (see, e.g., Clackson, Mattos et al., Hubbard, Cunningham et al., Kubinyi, Kleinberg et al., all herein incorporated by reference).

In another embodiment, potential inhibitors of a regulon target gene can be identified by the following steps:

5
a) creating a host cell in which the target gene has been altered or inactivated by mutation;

b) comparing gene expression profiles in the mutated host cell to those in a host cell which expresses the normal target gene;

c) identifying one or more potential target-dependent reporter genes

10
whose expression is altered in the host cell in which the target gene has been altered or inactivated compared to the host cell which expresses the normal target gene; and

d) screening one or more compounds for their effects on expression of the target-dependent reporter gene.

15
If expression of the target-dependent reporter gene increases in the host cell harboring an altered or inactivated target gene, then a potential inhibitor of the regulon target gene will increase expression of the target-dependent reporter gene, and if expression of the target-dependent reporter gene decreases in the host cell harboring an altered or inactivated target gene, then a potential inhibitor of the regulon target

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gene will decrease expression of the target-dependent reporter gene.

The method may further comprise the step, performed before step d), of assessing the specificity of a potential target-dependent reporter gene by comparing gene expression profiles the potential target-dependent reporter gene to a plurality of genes in a database of compiled gene expression profiles to generate individual

25
expression correlation coefficients wherein a target-dependent reporter gene whose expression correlates with the expression of the regulon target gene and with a minimal number or no other gene is selected over one whose expression correlates with a greater number of genes based on expression correlation coefficients. The method may also encompass upstream sequences that control expression of the target-

30
dependent reporter genes fused to a heterologous coding sequence, and the fusion is

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used to screen compounds for potential inhibitors of the regulon target gene, as discussed above.

In a preferred embodiment, the target gene is selected from *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

Pharmaceutical Applications

Compounds that bind to target proteins or regulate target gene expression can be tested in yeast cell systems and heterologous host cell systems (e.g., human cells) to verify that they do not have undesirable side effects. In addition, the yeast GRM can be used to make sure that the compounds do not adversely alter gene transcription (e.g., in an undesirable way). Of course, certain changes in gene expression may be inevitable and many of these will not be deleterious to the patient or host organism. Once lead compounds have been identified, these compounds can be refined further via rational drug design and other standard pharmaceutical techniques.

The compounds of this invention may be formulated into pharmaceutical compositions and administered *in vivo* at an effective dose to treat a particular disease or condition. Determination of a preferred pharmaceutical formulation and a therapeutically efficient dose regiment for a given application is within the skill of the art taking into consideration, for example, the condition and weight of the patient, the extent of desired treatment and the tolerance of the patient for the treatment.

Administration of the compounds of this invention, including isolated and purified forms, their salts or pharmaceutically acceptable derivatives thereof, may be accomplished using any conventionally accepted mode of administration.

The pharmaceutical compositions of this invention may be in a variety of forms, which may be selected according to the preferred modes of administration. These include, for example, solid, semi-solid and liquid dosage forms such as tablets, pills, powders, liquid solutions or suspensions, suppositories, and injectable and infusible solutions. The preferred form depends on the intended mode of

administration and therapeutic application. Modes of administration may include oral, parenteral, subcutaneous, intravenous, intralesional or topical administration.

The compounds of this invention may, for example, be placed into sterile, isotonic formulations with or without cofactors which stimulate uptake or

5   stability. The formulation is preferably liquid, or may be lyophilized powder. For example, the inhibitors may be diluted with a formulation buffer comprising 5.0 mg/ml citric acid monohydrate, 2.7 mg/ml trisodium citrate, 41 mg/ml mannitol, 1 mg/ml glycine and 1 mg/ml polysorbate 20. This solution can be lyophilized, stored under refrigeration and reconstituted prior to administration with sterile Water-For-Injection

10   (USP).

Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical administration, including those for inhalation, may be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized powder compositions, the active

15   ingredient in finely divided form may be used in admixture with a larger-sized pharmaceutically acceptable inert carrier comprising particles having a size, for example, of up to 100 micrometers in diameter. Alternatively, the composition may be pressurized and contain a compressed gas, such as nitrogen or a liquified gas propellant. The liquified propellant medium and indeed the total composition is

20   preferably such that the active ingredient does not dissolve therein to any substantial extent.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions

25   with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, ear drops, eye ointments, powders and solutions are also contemplated as being within the scope of this invention.

The pharmaceutical compositions of this invention may also be administered using microspheres, microparticulate delivery systems or other sustained

30   release formulations placed in, near, or otherwise in communication with affected

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tissues or the bloodstream. Suitable examples of sustained release carriers include semipermeable polymer matrices in the form of shaped articles such as suppositories or microcapsules. Implantable or microcapsular sustained release matrices include polylactides (U.S. Patent No. 3,773,319; EP 58,481), copolymers of L-glutamic acid

5   and gamma ethyl-L-glutamate (Sidman et al., 1985); poly(2-hydroxyethyl-methacrylate) or ethylene vinyl acetate (Langer et al., 1981, Langer, 1982).

The compounds of this invention may also be attached to liposomes, which may optionally contain other agents to aid in targeting or administration of the compositions to the desired treatment site. Attachment of the compounds to

10   liposomes may be accomplished by any known cross-linking agent such as heterobifunctional cross-linking agents that have been widely used to couple toxins or chemotherapeutic agents to antibodies for targeted delivery. Conjugation to liposomes can also be accomplished using the carbohydrate-directed cross-linking reagent 4-(4-maleimidophenyl) butyric acid hydrazide (MPBH) (Duzgunes et al., 1992), herein

15   incorporated by reference.

Liposomes containing pharmaceutical compounds may be prepared by well-known methods (See, e.g. DE 3,218,121; Epstein et al., 1985; Hwang et al.,1980; U.S. Patent Nos. 4,485,045 and 4,544,545). Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than

20   about 30 mol.% cholesterol. The proportion of cholesterol is selected to control the optimal rate of MAG derivative and inhibitor release.

The compositions also will preferably include conventional pharmaceutically acceptable carriers well known in the art (see, e.g., Remington's Pharmaceutical Sciences, 16th Edition, 1980, Mac Publishing Company). Such

25   pharmaceutically acceptable carriers may include other medicinal agents, carriers, genetic carriers, adjuvants, excipients, etc., such as human serum albumin or plasma preparations. The compositions are preferably in the form of a unit dose and will usually be administered one or more times a day.

EXAMPLE 1: PREPARATION OF THE Genome Reporter Matrix™

Construction of Reporter Gene Fusions (Method 1)

The regulatory region of each yeast gene was cloned into one of two vectors, pAB1 or pAB2. The vector pAB1 was constructed in the following manner:
5 First, the polymerase chain reaction (PCR) was used to amplify the transcriptional terminator region from the gene PGK1 using the oligonucleotides 5P-PGKTERM (5'-GATTGAATTCAATTGAAATCGATAG-3') and 3P-PGKTERM (5'-CCGAGGCGCCGAATTTTCGAGTTAT-3'). The amplified fragment consists of the 263 base-pair region immediately downstream of the PGK1 stop codon, and contains
10 an EcoRI site at the 5' end and a NarI site at the 3' end. These restriction sites were engineered into the two PCR primers (underlined sequences). The terminator was then cloned into YIplac211 that had been linearized with EcoRI and NarI, yielding pAB34. Next, the coding region of the green fluorescent protein (GFP) from *Aequoria victoria* was amplified by PCR using the oligonucleotides 5P-GFP-ORF (5'-
15 CATGTCTAGAGGAGAAGAACTTTTC-3') and 3P-GFP-ORF (5'-CGCGAATTCCTATTTGTATAGTTCA-3'). Again, these oligonucleotides contain engineered XbaI and EcoRI sites at the 5' and 3' ends, respectively (underlined). This fragment was cloned into pAB34, linearized with XbaI and EcoRI, to produce pAB35. Finally, the GFP-PGK terminator fragment was moved into the episomal vector
20 YEplac195 (9) as an XbaI/NarI fragment, thereby producing pAB1.

The vector pAB2 is pAB1 with an altered multiple cloning site (MCS). The new MCS contains 8 basepair recognition sites for three restriction enzymes. These larger 8 base-pair recognition sites occur less frequently throughout the yeast genome than the 6 base-pair sites present in the MCS of pAB1. Thus, the utilization of
25 restriction enzymes that recognize 8 base-pair sequences to clone the various regulatory regions (engineered into the PCR primers used to amplify the regions) would minimize the occurrence of those sites within the regions themselves. To construct pAB2, pAB1 was linearized with XbaI and SphI, dropping out the existing MCS, and an adapter containing the new MCS was ligated in. The adapter was made
30 by hybridizing two oligonucleotides, 8Cutter (5'-

CGGCGCGCCGCGGCCGCATGGCCGGCCAAT-3') and 8CutEnd (5'-

CTAGATTGGCCGGCCATGCGGCCGCGGCGCGCCGCATG-3'). This adapter has

sites for the restriction enzymes FseI, NotI, and AscI (underlined).

The promoter regions were cloned utilizing PCR of genomic DNA

5   prepared from a strain derived from S288c; JRY147 (MATa SUC2 mal mel gal2

CUP1). The promoter-specific primers were designed such that the proximal primer

spanned the start codon of the specific gene and included a few (usually four) codons

derived from the gene. The position of the distal primer was determined on a case-by-

case basis depending on the distance to, and orientation of, the neighboring open

10   reading frame (ORF) and the restriction sites present. Where the upstream ORF was

positioned in a divergent orientation and within 1,200 base-pairs, the size of the

promoter fragment amplified was adjusted such that all nucleotides up to, but not

including, the start codon of the upstream ORF were present. In cases where the

upstream ORF was situated in the same orientation, the amplified fragment was

15   designed to extend into the coding region but not so as to include the start codon.

Both primers had restriction enzyme recognition sites engineered into the ends to allow

the subsequent cloning of the PCR fragment into pAB1, or pAB2.

Construction of Reporter Gene Fusions (Method 2)

In another method for constructing genome reporter constructs, a

20   vector comprising a marker gene having an amber mutation and a *supF* tRNA gene

which suppresses the amber mutation is used as the parent vector.

A plasmid cloning vector was constructed which comprises a mutant β-

lactamase gene with an amber mutation and a *supF* tRNA gene. Downstream of the

supF tRNA gene there is a "stuffer" DNA fragment which is flanked by BsmBI

25   restriction sites. The BsmBI restriction enzyme cuts outside of its six base pair

recognition sequence (see, e.g., New England Biolabs 96/97 Catalog, p. 23) and

creates a four nucleotide 5' overhang. When the plasmid cloning vector is digested

with BsmBI, the enzyme cleaved within the stuffer DNA and within the adjoining

tRNA gene and deleted the four 3' terminal nucleotides of the gene. The deleted *supF*

tRNA gene encodes a tRNA which cannot fold correctly and is non-functional, i.e., it could not suppress the amber mutation in the mutant β-lactamase gene (β-lactamase (amber)). Downstream from the stuffer DNA fragment is the coding region of a modified green fluorescent protein ("*GFP*") gene.

5   The stuffer DNA was excised from the vector by digestion with BsmBI. The double-stranded DNA at the *supF*-stuffer fragment junction, produced by BsmBI digestion, is shown below. The tRNA gene sequences are indicated in bold:

```
5'  ..supF..TC        CCCCGGAGACGTC..stuffer..
        ..AGGGGG           CCTCTGCAG..5'
10          BsmBI
```

The 3' terminal sequence of the *supF* gene necessary for proper function is TCCCCCACCA. The vector, once cleaved with BsmBI, lacks the *supF* tRNA **ACCA** terminal nucleotides if the overhangs self-anneals during re-circularization of the plasmid in the absence of insert.

15   A DNA insert containing the upstream regulatory sequence from a yeast ORF was generated as a PCR fragment. Two oligonucleotides were designed to flank the DNA insert sequences of interest on a template DNA and anneal to opposite strands of the template DNA. These oligonucleotides also contained a sequence at their respective 5' ends that, when converted into a 5' overhang (in the double-stranded

20   PCR fragment generated using the oligonucleotides), is complementary to the overhangs on the cloning vector generated by BsmBI endonucleolytic cleavage.

Oligonucleotide #1 comprises the 5' terminal sequence: 5' CCCCACCA .... The remaining nucleotides 3' to this sequence were designed to anneal to sequences at one end of the DNA insert of choice, in this Example, to one of a

25   multitude of yeast expression control sequences.

As highlighted in bold above, oligonucleotide #1 comprises the base pairs needed to restore the wild-type 3' terminal end of the *supF* tRNA gene. These base pairs are located immediately 3' to the sequence that allows the insert to anneal to the overhang in the BsmBI-digested pAB4 vector.

30   Oligonucleotide #2 comprises the 5' terminal sequence: 5' TCCTG .... The remaining nucleotides 3' to this sequence were designed to anneal to sequences at

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the other end of the DNA insert of choice, in this Example, to one of a variety of yeast expression control sequences which may be used according to this invention.

The DNA template (*S. cerevisiae* genomic DNA) and the two oligonucleotides were annealed and the hybrids were amplified by polymerase chain reaction using Klentaq™ polymerase and PCR buffer according to the manufacturer's instructions (Clontech). Briefly, 15 ng *S. cerevisiae* genomic DNA served as template DNA in a 10µl PCR reaction containing 0.2mM dNTPs, PCR buffer, Klentaq™ polymerase, and 1 µL of an 8µM solution containing the primer pairs. The PCR reaction mixture was subjected to the following steps: a) 94ᵒC for 3 min; b) 94ᵒC for 15 sec; c) 52ᵒC for 30 sec; d) 72ᵒC for 1 min, 45 sec; and e) 4ᵒC indefinitely. Steps b) through d) were repeated for a total of 30 cycles. The PCR amplification product was purified away from other components of the reaction by standard methods.

To generate the desired 5' overhangs on the ends of the PCR amplification product, the PCR fragment was treated with DNA polymerase I in the presence of dTTP and dCTP. Under these conditions, DNA polymerase I fills in 3' overhangs with its 5' to 3' polymerase activity and also generates 5' overhangs with its 3' to 5' exonucleolytic activity, which, in the presence of excess dTTP and dCTP, removes nucleotides in a 3' to 5' direction until a thymidine or a cytosine, respectively, is removed and then replaced.

The overhangs generated by this reaction are:

a)   At the 5' end (*supF* tRNA restoring end) of the DNA insert:

```
5' CCCCACCA..        becomes      5' CCCCACCA..
        GGGGTGGT..                             TGGT..
```

b)   At the 3' end of the DNA insert (joined to the *GFP* coding sequence):

```
5' CAGGA..        becomes      5' C
   GTCCT..                        GTCCT..
```

This DNA insert, now comprising 5' overhangs compatible with one of each of the ends of the BsmBI-cleaved pAB4 vector, was used as substrate in a standard ligation reaction with the BsmBI-cleaved pAB4 vector. The resulting ligation

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mixture was used to transform competent *E. coli* cells. The cells were plated on agar plates in the presence of ampicillin.

Colonies that grew in the presence of ampicillin were producing functional β-lactamase enzyme and each harbored the desired recombinant DNA
5   molecule, having a DNA insert with a yeast expression control sequence inserted upstream of the modified *GFP* coding region. The *supF* gene on vectors which re-ligated without a DNA insert did not express a functional *supF* tRNA and did not make functional β-lactamase. Thus, they were not found in transformed host cells grown on ampicillin.

10   *Construction of Yeast Strains*

Strain ABY11 (MATa leu2Δ1 ura3-52) of *S. cerevisiae* was used. ABY11 is derived from S288c. GRM arrays were grown at 30°C on solid casamino acid medium (Difco) with 2% glucose and 0.5% UltraPure Agarose (Gibco BRL). The medium was supplemented with additional amino acids and adenine (Sigma) at the
15   following concentrations: adenine and tryptophan at 30 µg/ml; histidine, methionine, and tyrosine at 20 µg/ml; leucine and lysine at 40 µg/ml. Stock solutions of the supplements were made at 100x concentrations in water. Yeast cells were transformed with the reporter plasmids prepared by Method 1 or Method 2 (above) by the lithium acetate method (Ito et al., 1983, and Schiestl and Gietz, 1989).

20   *Determinations of Reporter Gene Expression Levels*

Solutions of test compounds were added directly to the yeast strains or were coated on plates prior to addition of the yeast strains. The individual strains comprising the GRM were maintained as independent colonies (and cultures) in a 96-well format, in medium selecting for the URA3-containing reporter plasmid. Prior to
25   each experiment, fresh dilutions of the reporter-containing strains were inoculated and grown overnight at 30°C. A Hamilton MicroLab 4200, a multichannel gantry robot equipped with a custom pin tool device capable of dispensing 50 nanoliter volumes in a highly reproducible manner, was used to array the matrix of yeast strains in a uniform

manner onto solid agar growth media at a density of 1536 reporter strains per 110 cm^2 plate. Fifty nanoliters of yeast liquid cultures arrayed onto solid medium by the Hamilton MicroLab 4200 results in colony-to-colony signal reproducibility of less than 5% variation. Once arrayed, each plate was grown at 30°C for 18 hours or at 25°C

5   for 24 hours.

The level of fluorescence expressed from each reporter gene fusion was determined using a Molecular Dynamics Fluorimager SI. AIS image analysis software (Imaging Research, Ontario CA) was used to quantitate the fluorescence of each colony in the images. Generally, the drug treatments were performed at several

10   concentrations, with the analysis based upon the concentration producing the most informative expression profile.

EXAMPLE 2: IDENTIFICATION OF *HES1* AS A REGULON INDICATOR GENE

The effects of Simvastatin on the Genome Reporter Matrix™ were

15   tested at a concentration of 20 μg/ml. The *HES1* reporter gene construct was induced by a natural log ratio of 4.2 (treated/untreated), indicating that the *HES1* reporter had an excellent signal to noise ratio induction in response to Simvastatin. The *HES1* gene encodes a protein with a significant amount of similarity with oxysterol binding proteins and has been implicated in isoprenoid metabolism (**Figure 35**). Analysis of

20   gene expression data with the Genome Reporter Matrix™ revealed that *HES1* expression is highly correlated with expression of genes encoding enzymes of the isoprenoid biosynthetic pathway (**Figure 36**).

The specificity of the *HES1* reporter for inhibitors of ergosterol biosynthesis was tested *in silico*. The expression of the *HES1* reporter was examined

25   in data from 710 experimental treatments of the Genome Reporter Matrix™. Basal levels of *HES1* reporter gene expression were 0.1 units. Units are defined as an arbitrary fluorescent value that has been normalized such that a value of 1.0 equals the mean reporter fluorescent level of all members of the Genome Reporter Matrix™ in a given experiment. All treatments (a total of 51) that induced *HES1* reporter gene

levels to 0.5 units or greater were treatments known to inhibit ergosterol biosynthesis, indicating a high degree of specificity for this pathway (**Figure 37**).

The utility of the *HES1* reporter gene in a high-throughput screen was tested by incubating a yeast strain harboring the *HES1* reporter in a 384-well array

5   containing various concentraions of ergosterol biosynthesis inhibitors (Econazole and Simvastatin) and nonspecific drugs (Flucytosine and Nifedipine). Cells were grown to mid-log phase at 30°C in casamino acids medium (0.67% yeast nitrogen base, 2% glucose, 2% casamino acids). Cell density was adjusted prior to incubation in various concentrations of drug. Arrays were incubated at 30°C for 24 hrs prior to imaging.

10   The *HES1* reporter was found to be specifically induced by Econazole and Simvastatin but not by Flucytosine or Nifedipine.

To further test the viability of this indicator gene in a high-throughput screen, the regulation of the *HES1* reporter was tested in two different strain backgrounds. ABY11 (*MATa leu2Δ1 ura3-52*) is a wild-type strain. ABY140

15   (*MATa his3Δ1 leu2Δ0 met15Δ0 pdr5::KanMX ura3Δ0 yor1::KanMX*) is a strain containing mutations in two multidrug resistance genes. Induction of the *HES1* reporter gene in ABY140 was found to be more sensitive to Simvastain and Econazole but not to Flucytosine or Nifedipine when compared to ABY11.

The ABY140 [*HES1*] strain was used to screen approximately 16,800

20   chemicals from a combinatorial chemistry library. One percent of these chemicals induced the *HES1* indicator gene. Twenty-four of these chemical were further tested in a secondary screen for the ability to induce four additional indicator (also referred to as reporter) genes whose expression are also coordinately regulated with genes encoding ergosterol biosynthetic enzymes. Eight of these twenty-four chemicals also

25   induced these reporter genes, suggesting that these chemicals interfere with ergosterol biosynthesis.

This example reveals how a high quality promoter sequence identified from systematic genome expression data can be employed with a significant degree of confidence to identify chemicals with a desired biological activity.

The DNA and amino acid sequence of *HES1* is shown in **Figures 62 and 63**, respectively.

EXAMPLE 3: IDENTIFICATION OF *YJL105w* AS A TARGET GENE

YJL105w was a previously uncharacterized ORF which contains a PHD

5   finger suggesting that it functions as a transcription factor (**Figure 1**). Gene
expression correlation coefficients were calculated for 1532 reporter constructs
including known genes involved in sterol biosynthesis. Several uncharacterized genes,
including *YJL105w*, were found to have highly correlated gene expression with genes
encoding sterol biosynthetic enzymes. *YJL105w* expression correlated very well (0.83)

10   with expression of *CYB5*, a gene involved in ergosterol biosynthesis (**Figure 2**).
Cyb5p is thought to be an electron donor for sterol modifying enzymes (Mitchell A.G.,
Martin C.E., *J. Biol. Chem.*, 1995, **270**(50):29766-72). Expression of *YJL105w* was
induced considerably by drugs that inhibit sterol biosynthesis as well as by a mutation
in the gene encoding HMG-CoA Synthase (**Figure 3**). The *YJL105w* reporter

15   construct comprises 1200 base-pairs of DNA sequence 5' to the ATG start codon and
thus, contains sequence information sufficient to confer the observed regulated
expression.

To test whether *YJL105w* has a role in isoprenoid metabolism, a
yjl105w mutant where the entire ORF was replaced with the kanamycin resistance gene

20   was constructed. Approximately 5×10^6 cells of the *yjl105w* mutant strain and a wild-
type control strain (ABY363, MATα *his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) were plated
onto separate non-selective agar plates. The sterol biosynthetic inhibitor lovastatin
(250μg) was applied to a sterile disk on each lawn and the cells were allowed to grow
overnight at 30°C. The *yjl105w* mutant strain was found to be significantly more

25   resistant to lovastatin treatment, further implicating this ORF in lipid metabolism
(**Figure 4**).

YJL105w appears to be fungal-specific since no apparent mammalian
counterparts were found. Although *YJL105w* is not an essential gene, it could provide
utility for constructing strains for specific applications. For instance, the resistance to

lovastatin conferred by a *yjl105w* mutant could result from an elevated flux through the isoprenoid biosynthetic pathway. Such a condition may result from an altered composition of the cell's lipid bilayer that triggers the induction of synthesis of isoprenoid biosynthetic enzymes and/or reduces the cell's permeability to lovastatin. In either of these cases, a strain defective for *YJL105w* could be useful for constructing strains that could grow under extreme situations, such as in industrial applications. Examples of extreme conditions include growth at high or low temperatures (>35°C or <20°C) or in osmotically stressful conditions or in the presence of amphipathic solutes. Alternatively, the resistance to lovastatin in the *yjl105w* mutant could result from decreased expression of membrane transporters or channels that allow entry of foreign compounds (xenobiotics). In this case, overexpression of *YJL105w* could produce a highly permeablized strain that would have numerous applications where entry of compounds into a cell is limited by permeability or availability of compounds. A mammalian counterpart of this ORF, if found, could be useful as a diagnostic marker for people with high serum cholesterol levels. Individuals that have mutations, null or weak (hypomorphic) alleles, might be expected to have a higher rate of sterol synthesis.

The DNA and protein sequences of *YJL105w* are depicted in Figures 39 and 40, respectively.

EXAMPLE 4: IDENTIFICATION OF *YMR134w* AS A TARGET GENE

YMR134w is an ORF that had been suggested previously to be involved in iron metabolism (**Figure 5**). Among 1532 reporter constructs, *YMR134w* expression was found to be highly correlated with the expression of *ERG2* (**Figure 6**) and is therefore likely to be involved in lipid metabolism. The *YMR134w* reporter construct was found to be highly induced by various statins (inhibitors of HMG-CoA reductase) and azole compounds (inhibitors of lanosterol 14-alpha demethylase, *ERG11*) (**Figure 7**). The *YMR134w* reporter construct comprises 1200 base-pairs of DNA sequence 5' to the ATG start codon and thus, contains sequence information sufficient to confer the observed regulated expression. A database search for

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YMR134w-related protein sequences revealed a weak similarity to human vascular endothelial growth factor receptor **(Figure 8)**.

The DNA and protein sequences of *YMR134w* are depicted in **Figures 41 and 42**, respectively.

5   **EXAMPLE 5: IDENTIFICATION OF *YER044c* AS A TARGET GENE**

YER044c was a previously uncharacterized yeast ORF with one predicted transmembrane domain **(Figure 9)**. *YER044c* expression is significantly correlated with the expression of *ERG2* (0.82, **Figure 10**). Statins, azoles and a deletion mutant of the *ERG11* gene each induce expression of the *YER044c* reporter

10   construct most significantly in 498 treatments of the GRM **(Figure 11)**. The *YER044c* reporter construct comprises 1200 base-pairs of DNA sequence 5' to the ATG start codon and thus contains sequence information sufficient to confer the observed regulated expression. DNA and proteins sequence database comparisons with the predicted protein sequence of *YER044c* revealed an apparent *Schizosaccharomyces*

15   *pombe* counterpart and numerous mammalian EST apparent counterparts **(Figures 12-14)**.

The DNA and protein sequences of *YER044c* are depicted in **Figures 43 and 44** respectively. The apparent mouse, human and rat EST counterparts of *YER044c* are depicted in **Figures 45-47**, respectively.

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EXAMPLE 6: IDENTIFICATION OF *YLR100w* AS A TARGET GENE

YLR100w was a previously uncharacterized yeast ORF **(Figure 15)**. Expression of *YLR100w* correlated significantly (0.82) with *CYB5* in the GRM composed of 6036 reporter constructs in 706 experimental treatments. The correlation

25   of expression of *YLR100w* to the expression of *CYB5* implied a role of *YLR100w* in lipid metabolism. Expression of the *YLR100w* reporter was induced significantly by statins, azoles and in a yeast *erg11* mutant consistent with a role of *YLR100w* in lipid metabolism **(Figure 17)**. Searches of DNA and protein sequence databases for similar

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sequences revealed a GenBank entry for a 17-beta-hydroxysteroid dehydrogenase mouse cDNA (**Figure 18**).

The sequence of the mouse cDNA is shown in **Figure 53**. Given the protein sequence similarity (**Figure 19**) and the fact that yeast is not known to

5   synthesize steroid hormones, it is conceivable that the mouse cDNA encodes a protein with another role in lipid metabolism. In this case, the mammalian protein could have utility as a pharmacological target to modulate lipid metabolism. Another GenBank entry was found for a rat ovarian specific protein with significant similarity to *YLR100w*. The sequence of the rat protein is shown in **Figure 65**. Two mouse ESTs

10   were found to be significantly similar to *YLR100w*. The sequence of the two mouse ESTs are shown in **Figures 51 and 52**. A human EST was found that was similar to *YLR100w*, but to a lesser extent than the two mouse ESTs.

The DNA and protein sequences of *YLR100w* are depicted in **Figures 48 and 49**, respectively. The sequence of the human EST is shown in **Figure 50**.

15   **EXAMPLE 7:   IDENTIFICATION OF *YER034w* AS A TARGET GENE**

YER034w is a yeast ORF that had been shown previously not to be essential for cell viability (**Figure 20**). Expression of the *YER034w* reporter construct was found to be correlated (0.75) with the expression of a *GPA2* reporter construct in a GRM composed of 1532 reporters treated under 498 experimental conditions

20   (**Figure 21**). *GPA2* encodes the alpha subunit of a trimeric G protein involved in pseudohyphal differentiation (Lorentz, M.C. and Heitman, J. *EMBO J.* 1997 16:7008-7018). This correlation suggested that *YER034w* had a role in the pseudohyphal growth and could represent a new antifungal target.

To test this hypothesis, a diploid homozygous *yer034w* knockout strain

25   was purchased from Research Genetics (Huntsville, AL). Wild-type cells (ABY13, *MATa/MATalpha his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 met15Δ0/MET15 LYS2/lys2Δ0 ura3Δ0/ura3Δ0*) and the homozygous *yer034w* knockout strain were plated onto low nitrogen plates to stimulate pseudohyphal differentiation. After four days at 25°C, plates were examined under a microscope. The *yer034w* knockout strain had

undergone significantly more differentiation than the wild-type control both in terms of numbers of projections per colony (**Figure 22**) and the size of the hyphae. This result implicated *YER034w* in the dimorphic transition of cells from yeast to pseudohyphae. The ability of fungi to undergo this morphological transition has been suggested to be a

5   critical aspect of fungal pathogenicity. A search for related mammalian protein sequences did not identify any obvious counterparts suggesting that this protein is fungal-specific and may be an amenable anti-fungal target.

The DNA and protein sequences of YER034w are depicted in **Figures 54 and 55**, respectively.

10   **EXAMPLE 8: IDENTIFICATION OF *YKL0774w* AS A TARGET GENE**

YKL077w was a previously uncharacterized ORF with one predicted transmembrane domain (**Figure 23**). Expression of the *YKL077w* reporter construct was found to be correlated (0.92) with the expression of a *SGV1* reporter construct in a GRM composed of 1532 reporters treated under 498 experimental conditions

15   (**Figure 24**). Sgv1p is a Cdc28p-related protein kinase that is essential for cell viability. In addition to Sgv1p expression, *YKL077w* expression correlated highly (>0.8) with *PKC1* and *RHO1* (**Figure 25**), genes involved in cell wall integrity and cytoskeletal reorganization. Database searches with the predicted protein sequence of *YKL077w* did not identify apparent mammalian counterparts (**Figure 26**). *YKL077w*

20   could represent an antifungal target given the lack of a mammalian homolog and its proposed involvement in cellular structure and/or proliferation. Nevertheless, in the event a mammalian counterpart is discovered, it could represent an anti-proliferative target as well.

The DNA and protein sequences of *YKL077w* are depicted in **Figures**

25   **56 and 57**, respectively.

EXAMPLE 9: IDENTIFICATION OF *YGR046w* AS A TARGET GENE

YGR046w was a previously uncharacterized yeast ORF that has been shown to be essential for viability (**Figure 27**). Expression of *YGR046w* correlated

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significantly (0.90) with *IRA2* in the GRM composed of 6036 reporter constructs in 706 experimental treatments **(Figure 28)**. Ira2p is a GTPase activating protein (GAP) for Ras1p and Ras2p. In addition to *IRA2* expression, *YGR046w* expression correlated very well (>0.77) with the expression of known genes involved cell proliferation

5   functions **(Figure 29)**. The expression of *YGR046w* was found to be most sensitive to agents that disrupt mitochondrial function, create oxidative stress and disrupt the cytoskeleton **(Figure 30)**.

Given its proposed involvement in cell proliferation, *YGR046w* could represent a target for modulation of cell growth. A search of protein and DNA

10   sequence databases did not reveal any apparent mammalian homologs. Nevertheless, if such a sequence is identified, it may represent an anti-proliferative mammalian target.

The DNA and protein sequences of *YGR046w* are depicted in **Figures 58 and 59**, respectively.

EXAMPLE 10: IDENTIFICATION OF *YJR041c* AS A TARGET GENE

15   Mutant strains defective for *YJR041c* have been shown previously to display a severe growth defect, but no function for *YJR041c* was known **(Figure 31)**. Expression of *YJR041c* correlated significantly (0.83) with *MED7* in the GRM composed of 6036 reporter constructs in 706 experimental treatments **(Figure 32)**. Med7p encodes a component of the mediator complex involved in RNA polymerase II

20   transcription. *YJR041c* expression was also found to correlate significantly (>0.71) with several genes involved in different aspects of RNA metabolism. These processes include RNA polymerase I and II transcription, mRNA splicing, RNA turnover and ribosome function **(Figure 33)**.

Database searches for related sequence identified similar sequences

25   from *Schizosaccharomyces pombe* **(Figure 34)**. No obvious mammalian counterparts were identified suggesting that *YJR041c* is a fungal-specific protein. Given these factors, *YJR041c* could represent an attractive target for antifungal therapy. In the event a mammalian counterpart is identified, it also could represent a target with utility for modulating cell proliferation.

The DNA and protein sequences of *YJR041c* are shown in **Figures 60 and 61**, respectively.

EXAMPLE 11: SCREENING ASSAY USING THE GENOME REPORTER MATRIX™ TO IDENTIFY TARGET INHIBITORS

5   A mutant or conditional allele of target yeast gene is produced as discussed above. The allele may be conditional either for function or expression. For instance, the conditional allele may be a temperature-sensitive allele of the target gene or the target gene may be operably linked to an inducible promoter for regulated expression. In a preferred embodiment, the target gene is operably linked to an

10   inducible promoter that permits expression anywhere between 0% and 500% of wild type expression. The target gene of interest is transfected and expressed in yeast cells of the GRM that have a functional deletion of the target gene of interest. The level of expression of the conditional allele is varied between 0% and 500% of wild type expression, and the expression of the reporter constructs of the GRM is measured in

15   response to the expression of the target gene. The expression of the reporter constructs is then correlated to the expression of the target gene. Thus, one can identify a subset of genes that are either induced or repressed by overexpression of the target gene.

          The yeast strains containing the subset of genes whose expression is

20   dependent upon overexpression, and thus the function of the essential gene, are then used to screen compounds that are potential target inhibitors. The yeast strains are incubated with the compounds. If a reporter gene in a particular yeast strain is induced by overexpression of the target gene, then potential inhibitors are screened for the ability to downregulate the reporter gene. Conversely, if a reporter gene is repressed

25   by overexpression of the target gene, then potential inhibitors are screened for the ability to upregulate the reporter gene. Potential inhibitors are screened for the ability to appropriately upregulate and downregulate a number of the genes whose expression is dependent upon expression or overexpression of the target gene. When potential target inhibitors are identified, these candidate compounds are tested for their ability to

inhibit the pathway that the target gene is part of. For instance, if the target gene is *YER034w*, then the inhibitor may be tested for antifungal activity.

If a target gene has a plant or animal counterpart, one may express the plant or animal counterpart in a yeast strain lacking the target gene to see if the plant

5   or animal counterpart can functionally substitute for the yeast gene. If it can, then the plant or animal counterpart can be used in the above example to screen for potential targets for either a plant or animal inhibitor. This is especially useful if the target gene has a mammalian counterpart. Similarly, even if a plant, animal or mammalian counterpart has not been identified, potential inhibitors may be tested for their ability to

10   inhibit the pathway that the target gene is part of, if that pathway is shared by yeast and higher eukaryotes.

EXAMPLE 12: SIMULTANEOUS TRACKING OF MULTIPLE REPORTERS AS REGULON INDICATOR GENES

The effects of inactivating an osmotic stress pathway were tested by

15   deleting a pathway component (Hog1p stress-activated protein kinase). Using the *hog1* knock-out profile as model, multiple RIGs that would specifically indicate pathway inhibitors were identified and tested *in silico* by examining all conditions in which selected RIGs were activated or repressed. It was determined that simultaneously monitoring up-regulation of *PGU1* and down-regulation of *DAK1* gave

20   good specificity for pathway inactivation as determined by the separation of the hog1 knock-out profile from all other conditions in which these two reporters were affected (**Figure 74**). In this example, RIGs were not part of the target regulon but were chosen empirically based on behavior under all conditions.

Similarly, 2 RIGs were identified that could specifically indicate

25   mitochondrial inactivation by comparing the behavior these RIGs in the subset of treatments that target mitochondria with all treatments that affect these RIGs. It was determined that simultaneously measuring up-regulation of 2 RIGs (*STE18* and *YGL198w*) provides good specificity for mitochondrial perturbations as determined by

the separation of this subset of common treatments from all other conditions that affect these RIGs (**Figure 75**).

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

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CLAIMS

We claim:

1. A method for placing Gene X, a gene of unknown function, into a functional genetic group comprising the steps of:

5
   a) generating a gene expression profile for Gene X;

   b) comparing the gene expression profile of Gene X with gene expression profiles of a plurality of other genes in a database of compiled gene expression profiles to generate expression correlation coefficients;

10
   c) identifying based on their expression correlation coefficients a set of genes comprising Gene X that are coordinately expressed;

   d) determining if the one or more genes whose expression is most highly correlated with that of Gene X belong to a gene regulon involved in a known biological pathway, or a common set of

15
   biological reactions or functions; and

   e) optionally testing the effect on Gene X expression of at least one altered condition or treatment known to affect the function to which Gene X hs been ascribed;

   wherein Gene X is placed in the gene regulon of d) if Gene X expression is

20 coordinate with expression of that regulon.

2. A method for identifying a regulon indicator gene in a database of compiled gene expression profiles, wherein expression of the regulon indicator gene correlates with the expression of at least one known gene in a group of coordinately expressed genes or provide a measure of the function of a biological process of

25 interest, the method comprising the steps of:

   a) comparing gene expression profiles of a plurality of genes in the database to generate expression correlation coefficients;

b)   identifying based on their relative expression correlation coefficients a set of genes that are coordinately expressed;

c)   selecting a set of genes from b) which comprises one or more genes known to function in a particular biological pathway, or a common set of biological reactions or functions;

d)   selecting a member of the set of c) having one or more of the following characteristics:

1)   its expression profile is sensitive to one or more stimuli;

2)   its expression profile exhibits a large dynamic range in response to one or more stimuli;

3)   its expression profile exhibits a rapid kinetic response to one or more stimuli;

4)   its expression profile is specific to a known biological pathway or a common set of biological reactions or functions;

5)   the regulon indicator gene does not contain sequences that are problematic for maintaining on plasmids when introduced into host cells.

3. The method of claim 2, wherein the regulon indicator gene is co-regulated with one or more genes in the group of coordinately expressed genes of c).

4. The method of claim 2, wherein the regulon indicator gene, upon expression, controls the expression of at least one other gene in the group of coordinately expressed genes of c).

5. The method of claim 2, wherein the regulon indicator gene is of previously unknown function.

6. A method for selecting a novel regulon target gene from a database of compiled gene expression profiles, comprising the steps of:

a)   comparing gene expression profiles of a plurality of genes in the database to generate expression correlation coefficients;

5   b)   identifying based on their expression correlation coefficients a set of genes that are coordinately expressed;

c)   selecting from b) a set of genes comprising one or more genes of unknown function and one or more genes known to function in a particular biological pathway, or a common set of biological

10   reactions or functions of interest;   .

d)   selecting from the set of c) at least one gene of unknown function, Gene X, as a novel regulon target gene; wherein Gene X is a gene whose expression profile closely correlates to the expression profiles of the one or more genes of the set of c)

15   known to function in the particular biological pathway, or common set of biological reactions or functions of interest.

7. The method of claim 6, further comprising the step of generating individual correlation coefficients between the gene expression profile of Gene X and a plurality of genes in the database to assess the selectivity of Gene X as a novel regulon

20   target gene.

8. The method of claim 6, further comprising the step of determining whether the protein encoded by Gene X exhibits substantial homology to a human, non-human mammal, avian, amphibian, fish, insect or plant protein.

9. The method of claim 8, wherein said determining comprises the

25   steps of hybridizing Gene X to genomic DNA from human, non-human mammal, avian, amphibian, fish, insect or plant cells or tissue under low stringency conditions.

89

10. The method of claim 8, wherein said determining comprises the steps of:

a) comparing the DNA sequence of Gene X to the DNA sequences from other organisms or

b) obtaining an amino acid sequence encoded by Gene X and comparing it to amino acid sequences from other organisms.

11. The method of any one of claims 8-10, wherein the DNA or amino acid sequences from other organisms are contained within a database, and wherein the DNA or amino acid sequence encoded by Gene X is compared to the DNA or amino acid sequences from other organisms using a computer algorithm.

12. The method of claim 11, wherein the computer algorithm is blastp, tblastn or another algorithm that utilizes string alignments.

13. The method of claim 6, further comprising the steps of:

a) disrupting the function of Gene X or its homolog in a yeast cell; and

b) identifying whether the function of Gene X is essential for yeast germination, vegetative growth, pseudohyphal or hyphal growth.

14. A method for identifying a potential inhibitor of a regulon target gene, comprising the steps of:

a)   incubating a polypeptide comprising an amino acid sequence encoded by a regulon target gene with a compound under conditions effective to promote specific binding between the polypeptide and the compound; and

b)   determining whether the polypeptide bound to the compound; wherein the compound is a potential inhibitor if the compound binds to the polypeptide.

15. The method of claim 14, wherein the polypeptide comprises the full-length amino acid sequence encoded by the regulon target gene.

16. The method of claim 14, wherein the polypeptide comprises a functional fragment of the amino acid sequence encoded by the regulon target gene.

5   17. The method of claim 14, wherein the polypeptide is a fusion protein comprising an epitope tag or reporter gene.

18. The method of claim 14, wherein the polypeptide is attached to a solid support surface and the compound is in mobile phase.

19. The method of claim 14, wherein the compound is attached to a

10   solid support surface and the polypeptide is in mobile phase.

20. The method of claim 14, wherein the compound is a library selected from the group consisting of a combinatorial small organic library, a phage display library and a combinatorial peptide library.

21. The method of claim 14, wherein said determining is performed by

15   ELISA, RIA or BiaCORE analysis.

22. The method of claim 14, wherein said determining is performed by high throughput screening.

23. The method of claim 14, further comprising the step, performed before step a), of expressing in a host cell a regulon target gene.

24. The method of claim 14, wherein the target gene is selected from the group consisting of *YMR134w, YER034w, YJL105w, YKL077w, YGR046w, YJR041c, YER044c* and *YLR100w* and their mammalian homologs.

25. The method of claim 14, wherein the target gene is human EST W28235, a homolog of *YER044c*.

26. The method of claim 14, wherein the target gene is human EST R92053, a homolog of *YLR100w*.

27. The method of claim 14, wherein the target gene is mouse EST AI386195, a homolog of *YER044c*.

28. The method of claim 14, wherein the target gene is mouse EST AI226514, a homolog of *YLR100w*.

29. The method of claim 14, wherein the target gene is mouse EST AI528381, a homolog of *YLR100w*.

30. The method of claim 14, wherein the target gene is mouse gene 3319971, a homolog of *YLR100w*.

31. The method of claim 14, wherein the target gene is rat gene 1397235, a homolog of *YLR100w*.

32. The method of claim 14, further comprising performing, before step a), the step of expressing in a host cell a regulon target gene selected from the group consisting of *YMR134w, YER034w, YJL105w, YKL077w, YGR046w, YJR041c, YER044c* and *YLR100w* and their mammalian homologs.

33. A method for identifying a potential inhibitor of a regulon target gene, comprising the steps of:

a) creating a host cell in which the target gene has been altered or inactivated by mutation;

5

b) comparing gene expression profiles in the mutated host cell to those in a host cell which expresses the normal target gene;

c) identifying one or more potential target-dependent reporter genes whose expression is altered in the host cell in which the target gene has been altered or inactivated compared to the host

10

cell which expresses the normal target gene;

d) screening one or more compounds for their effects on expression of the target-dependent reporter gene;

wherein if expression of the target-dependent reporter gene increases in the host cell harboring an altered or inactivated target gene, then a potential inhibitor

15

of the regulon target gene will increase expression of the target-dependent reporter gene, and if expression of the target-dependent reporter gene decreases in the host cell harboring an altered or inactivated target gene, then a potential inhibitor of the regulon target gene will decrease expression of the target-dependent reporter gene.

34. The method of claim 33, further comprising the step, performed

20

before step d), of assessing the specificity of a potential target-dependent reporter gene by comparing gene expression profiles the potential target-dependent reporter gene to a plurality of genes in a database of compiled gene expression profiles to generate individual expression correlation coefficients wherein a target-dependent reporter gene whose expression correlates with the expression of the regulon target gene and with a

25

minimal number or no other gene is selected over one whose expression correlates with a greater number of genes based on expression correlation coefficients.

35. The method of claim 33 or 34, wherein upstream sequences that control expression of the target-dependent reporter gene are fused to a heterologous

coding sequence and that fusion used to screen compounds for potential inhibitors of the regulon target gene.

36. The method of claim 35, wherein the heterologous sequence comprises an epitope tag or a reporter gene.

5   37. The method of claim 35, wherein the fusion polypeptide is attached to a solid support surface and the compound is in mobile phase.

38. The method of claim 35, wherein the compound is attached to a solid support surface and the fusion polypeptide is in mobile phase.

39. The method of claim 33, wherein the compound is a library

10   selected from the group consisting of a combinatorial small organic library, a phage display library and a combinatorial peptide library.

40. The method of claim 33, wherein said screening is performed by ELISA, RIA or BiaCORE analysis.

41. The method of claim 33, wherein said screening is performed by

15   high throughput screening.

42. The method of claim 33, wherein the target gene is selected from the group consisting of *YMR134w*, *YER034w*, *YJL105w*, *YKL077w*, *YGR046w*, *YJR041c*, *YER044c* and *YLR100w* and their mammalian homologs.

44. The method of claim 33, wherein the target gene is human EST

20   W28235, a homolog of *YER044c*.

45. The method of claim 33, wherein the target gene is human EST R92053, a homolog of *YLR100w*.

46. The method of claim 33, wherein the target gene is mouse EST AI386195, a homolog of *YER044c*.

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47. The method of claim 33, wherein the target gene is mouse EST AI226514, a homolog of *YLR100w*.

48. The method of claim 33, wherein the target gene is mouse EST AI528381, a homolog of *YLR100w*.

49. The method of claim 33, wherein the target gene is mouse gene

10   3319971, a homolog of *YLR100w*.

50. The method of claim 33, wherein the target gene is rat gene 1397235, a homolog of *YLR100w*..

51. A method for inhibiting the expression of a regulon target gene in a host cell comprising the step of introducing into the host cell an inhibitor made

15   according to any one of claims   .

52. The method of claim 51, wherein the target gene is selected from the group consisting of *YMR134w, YER034w, YJL105w, YKL077w, YGR046w, YJR041c, YER044c* and *YLR100w* and their mammalian homologs.

53. An antisense oligonucleotide comprising a sequence

20   complementary to the sequence of an mRNA of a regulon target gene and effective to decrease transcription or translation of the gene.

54. The antisense oligonucleotide of claim 53 complementary to the sequence of the mRNA of a target gene selected from the group consisting of *YMR134w, YER034w, YJL105w, YKL077w, YGR046w, YJR041c, YER044c* and *YLR100w* and their mammalian homologs.

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55. A ribozyme comprising a sequence complementary to the sequence of an mRNA of a regulon target gene and effective to decrease transcription or translation of the gene.

56. The ribozyme of claim 55 complementary to the sequence of the mRNA of a target gene selected from the group consisting of *YMR134w, YER034w,*

10 *YJL105w, YKL077w, YGR046w, YJR041c, YER044c* and *YLR100w* and their mammalian homologs.

57. A neutralizing antibody to a protein encoded by a regulon target gene of a yeast or its mammalian homolog.

58. The neutralizing antibody of claim 57, wherein the target gene is selected from

15 the group consisting of *YMR134w, YER034w, YJL105w, YKL077w, YGR046w, YJR041c, YER044c* and *YLR100w* and their mammalian homologs.

59. A fusion protein comprising an amino acid sequence encoded by a regulon target gene of a yeast or its mammalian homolog and further comprising an epitope tag or a reporter gene.

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60. The fusion protein of claim 59, wherein the target gene is selected from the group consisting of *YMR134w, YER034w, YJL105w, YKL077w, YGR046w, YJR041c, YER044c* and *YLR100w* and their mammalian homologs.

61. A method for identifying a gene regulated by a regulon target gene of a yeast or its mammalian homolog, comprising the steps of:

a) overexpressing the target gene in host cells of a matrix comprising a plurality of units of cells, the cells in each unit containing a reporter gene operably linked to an expression control sequence derived from a gene of a selected organism; and

b) identifying genes that are either induced or repressed by overexpression of the target gene.

62. The method according to claim 61, wherein the target gene is selected from the group consisting of *YMR134w, YER034w, YJL105w, YKL077w, YGR046w, YJR041c, YER044c* and *YLR100w* and their mammalian homologs.

63. A method for identifying a regulon indicator gene in a database of compiled gene expression profiles, wherein expression of the regulon indicator gene provides a measure of the function of a biological pathway or process of interest, the method comprising the steps of:

a) examining exemplary expression profiles in response to one or more chemical or genetic treatments which target the pathway or process of interest to generate reporter sensitivity data;

b) selecting a set of genes from a) which comprises one or more genes most significantly affected in response to the treatment or treatments; and

c) selecting at least one gene from b) whose expression profile is maximized for its specificity and sensitivity to the treatment or class of treatments in a) compared to its sensitivity to all other treatments in the database.

64. The method of claim 63, wherein the regulon indicator gene is co-regulated with one or more genes in the set of genes of a).

65. The method of claim 63, wherein the regulon indicator gene, upon expression, controls the expression of at least one other gene in the set of genes of a).

5

YJL105w

GenBank No.   1008286

Chromosome   X

Protein   559 amino acids

63,867 Daltons

Comments: contains a PHD finger

Figure 1.
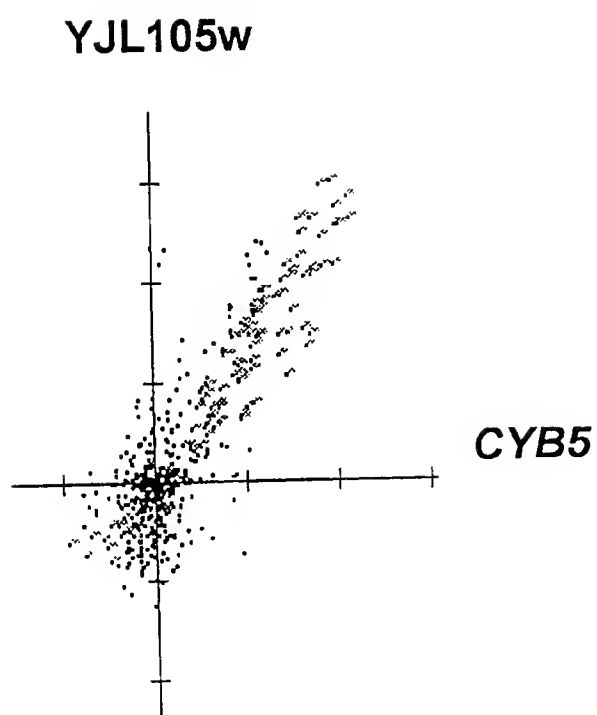
Figure 2.

Regulated Expression of YJL105w

Expt	Level	Natural Log Ratio	Treatment [baseline]
1455	9.1	+3.2	4.0ug/ml Fluvastatin - 18 hr [0.09]
1454	8.1	+3.1	8.0ug/ml Fluvastatin - 18 hr [0.13]
1537	7.9	+3.1	20ug/ml Lovastatin in 1 Ethanol - 18 hr [0.10]
1420	7.8	+3.1	20ug/ml Atorvastatin in 1 DMSO - 18 hr [0.14]
3455	7.8	+3.1	20ug/ml Lovastatin - 18 hr [0.20]
3456	7.8	+3.1	25ug/ml Lovastatin - 18 hr [0.20]
1944	6.5	+2.9	30ug/ml Mevastatin in 1.5 Ethanol - 18 hr [0.20]
1943	6.4	+2.9	15ug/ml Simvastatin in 1.5 Ethanol - 18 hr [0.13]
1554	5.8	+2.8	5ug/ml Simvastatin in 1 Ethanol - 18 hr [0.12]
1419	5.2	+2.7	30ug/ml Atorvastatin in 1 DMSO - 18 hr [0.12]
1553	5.1	+2.6	10ug/ml Simvastatin in 1 Ethanol - 18 hr [0.11]
3454	5.1	+2.6	10ug/ml Lovastatin - 18 hr [0.15]
1538	4.8	+2.6	10ug/ml Lovastatin in 1 Ethanol - 18 hr [0.09]
1421	4.4	+2.5	10ug/ml Atorvastatin in 1 DMSO - 18 hr [0.12]
1541	4.2	+2.4	10ug/ml Mevastatin in 1 Ethanol - 18 hr [0.08]
1456	4.1	+2.4	2.0ug/ml Fluvastatin - 18 hr [0.06]
1539	4.0	+2.4	5ug/ml Lovastatin in 1 Ethanol - 18 hr [0.08]
1540	4.0	+2.4	20ug/ml Mevastatin in 1 Ethanol - 18 hr [0.10]
2756	3.9	+2.4	[hmgs - ABY244.1 regulated (60)] - 18 hr [0.21]
2757	3.8	+2.3	[hmgs - ABY244.1 regulated (80)] - 18 hr [0.20]
2061	3.3	+2.2	35ug/ml Atorvastatin in 1 Ethanol - 18 hr [0.08]
1982	3.0	+2.1	0.125ug/ml Clotrimazole in 1 Methanol - 18 hr [0.19]
2060	2.9	+2.1	25ug/ml Atorvastatin in 1 Ethanol - 18 hr [0.07]
1542	2.8	+2.0	5ug/ml Mevastatin in 1 Ethanol - 18 hr [0.08]
1999	2.7	+2.0	20ug/ml Atorvastatin in 1 Ethanol - 18 hr [0.08]
3279	2.7	+2.0	0.15ug/ml Clotrimazole in 1 DMSO - 18 hr [0.13]
1935	2.6	+2.0	0.04ug/ml Econazole in 1 Methanol - 18 hr [0.18]
1478	2.5	+1.9	2.0ug/ml Fluconazole in 0.9 Saline - 18 hr [0.27]
1477	2.5	+1.9	3.0ug/ml Fluconazole in 0.9 Saline - 18 hr [0.31]
1983	2.5	+1.9	0.15ug/ml Clotrimazole in 1 Methanol - 18 hr [0.15]
3468	2.5	+1.9	20ug/ml Lovastatin [ABY139] - 18 hr [0.58]
2754	2.5	+1.9	[hmgs - ABY244.1 regulated (20)] - 18 hr [0.19]

Figure 3.
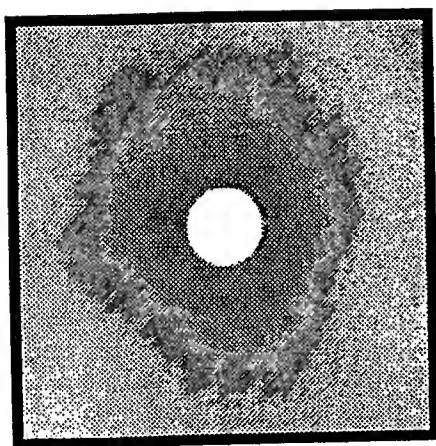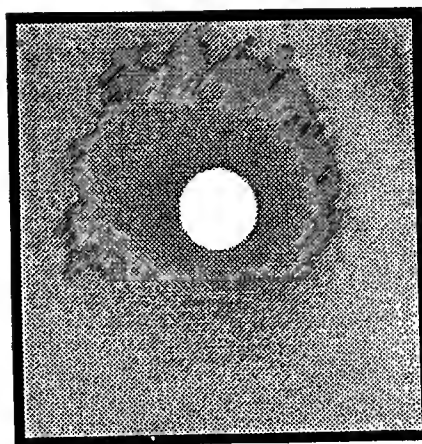
Wild-Type   *YJL105w* **Knockout**

Figure 4.

YMR134w

GenBank No.   606432

Chromosome   XIII

Protein   236 amino acids

27,911 Daltons

Comments: involved in iron metabolism; potential transmembrane domain

Figure 5.

YMR134w

ERG2
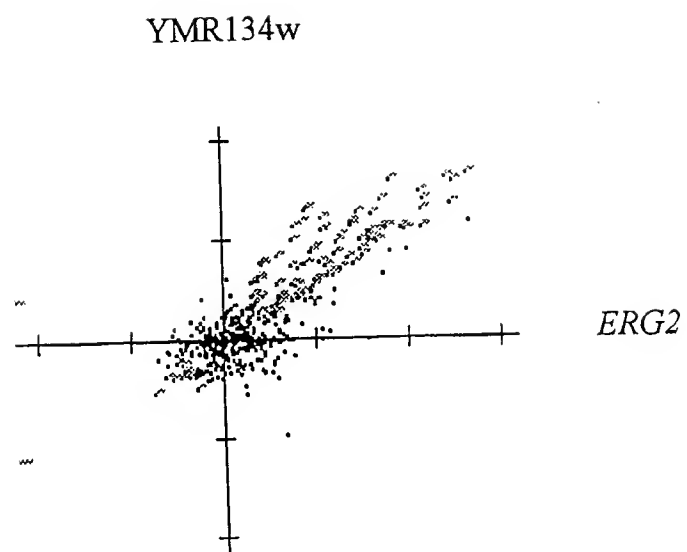
Figure 6.

Treatments Causing Highest Expression of YMR134w

Experiment Level log ratio   Treatment [baseline]

1943	1.3	+1.8	15ug/ml Simvastatin in 1.5 Ethanol - 18 hr [0.13]
1944	1.2	+1.7	30ug/ml Mevastatin in 1.5 Ethanol - 18 hr [0.20]
1419	1.2	+1.7	30ug/ml Atorvastatin in 1 DMSO - 18 hr [0.12]
1537	1.2	+1.7	20ug/ml Lovastatin in 1 Ethanol - 18 hr [0.10]
1454	1.2	+1.7	8.0ug/ml Fluvastatin - 18 hr [0.13]
1477	1.0	+1.5	3.0ug/ml Fluconazole in 0.9 Saline - 18 hr [0.31]
1553	0.9	+1.5	10ug/ml Simvastatin in 1 Ethanol - 18 hr [0.11]
1455	0.9	+1.5	4.0ug/ml Fluvastatin - 18 hr [0.09]
3455	0.9	+1.5	20ug/ml Lovastatin - 18 hr [0.20]
3456	0.9	+1.5	25ug/ml Lovastatin - 18 hr [0.20]
1538	0.9	+1.4	10ug/ml Lovastatin in 1 Ethanol - 18 hr [0.09]
3454	0.9	+1.4	10ug/ml Lovastatin - 18 hr [0.15]
1478	0.8	+1.4	2.0ug/ml Fluconazole in 0.9 Saline - 18 hr [0.27]
1540	0.8	+1.3	20ug/ml Mevastatin in 1 Ethanol - 18 hr [0.10]
1420	0.8	+1.3	20ug/ml Atorvastatin in 1 DMSO - 18 hr [0.14]
1611	0.8	+1.3	10ug/ml Fluconazole - 21 hr [0.04]
1554	0.7	+1.2	5ug/ml Simvastatin in 1 Ethanol - 18 hr [0.12]
3279	0.7	+1.2	0.15ug/ml Clotrimazole in 1 DMSO - 18 hr [0.13]
3469	0.7	+1.2	25ug/ml Lovastatin [ABY139] - 18 hr [0.57]
1605	0.7	+1.2	5ug/ml Fluconazole - 21 hr [0.04]
1936	0.7	+1.1	0.05ug/ml Econazole in 1 Methanol - 18 hr [0.14]
3468	0.7	+1.1	20ug/ml Lovastatin [ABY139] - 18 hr [0.58]

Figure 7.

Blastp search of GenBank

```
Sequences producing significant alignments:                          Score    E
                                                                     (bits)  Value

sp|P40207|YM17_YEAST   HYPOTHETICAL 27.9 KD PROTEIN IN REC114-PSO...   483   e-136
sp|P17948|VGR1_HUMAN   VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTO...    34   1.3
gi|3132831  (AF063657) vascular endothelial growth factor recept...    34   1.3
gi|2088746  (AF003142) contains similarity to C2H2-type zinc fin...    33   2.2
gi|886766   (U27832) Smt4p [Saccharomyces cerevisiae]                  32   2.9
sp|P40537|SMT4_YEAST   SMT4 PROTEIN >gi|1077779|pir||S49947 SMT4 ...    32   2.9
sp|P48034|ADO_BOVIN    ALDEHYDE OXIDASE >gi|1149575|emb|CAA60701| ...   32   2.9
sp|Q06278|ADO_HUMAN    ALDEHYDE OXIDASE >gi|2117502|pir||A49634 al...   32   5.0
```

tblastn search of dbest

```
                    No hits found
```

Figure 8.

YER044c

GenBank No.   603277

Chromosome   V

Protein   148 amino acids

                     17,140 Daltons

Comments: unknown function; potential transmembrane domain

Figure 9.

YER044c

ERG2
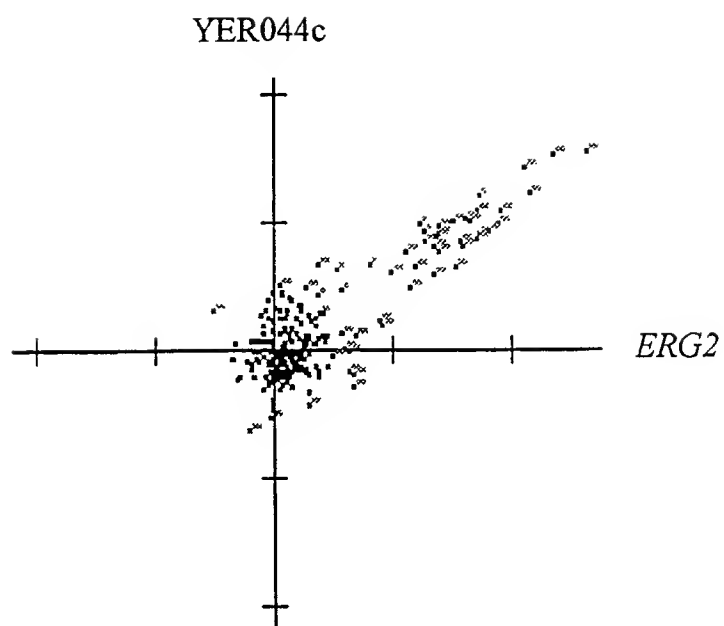
Figure 10.

Treatments Causing Highest Expression of YER044c

```
Experiment Level  log ratio     Treatment [baseline]
     1419    4.2     +1.7    30ug/ml Atorvastatin in 1 DMSO - 18 hr [0.12]
     1420    3.6     +1.5    20ug/ml Atorvastatin in 1 DMSO - 18 hr [0.14]
     1617    3.3     +1.4    20ug/ml Fluconazole - 21 hr [0.04]
     1454    3.2     +1.4    8.0ug/ml Fluvastatin - 18 hr [0.13]
     1537    3.1     +1.4    20ug/ml Lovastatin in 1 Ethanol - 18 hr [0.10]
     1943    3.0     +1.3    15ug/ml Simvastatin in 1.5 Ethanol - 18 hr [0.13]
     1623    3.0     +1.3    100ug/ml Fluconazole - 21 hr [0.04]
     3456    3.0     +1.3    25ug/ml Lovastatin - 18 hr [0.20]
     3455    3.0     +1.3    20ug/ml Lovastatin - 18 hr [0.20]
     1611    2.9     +1.3    10ug/ml Fluconazole - 21 hr [0.04]
     1553    2.7     +1.2    10ug/ml Simvastatin in 1 Ethanol - 18 hr [0.11]
     3454    2.5     +1.1    10ug/ml Lovastatin - 18 hr [0.15]
     1605    2.5     +1.1    5ug/ml Fluconazole - 21 hr [0.04]
     3279    2.5     +1.1    0.15ug/ml Clotrimazole in 1 DMSO - 18 hr [0.13]
     1455    2.4     +1.1    4.0ug/ml Fluvastatin - 18 hr [0.09]
     1669    2.4     +1.1    100ug/ml Fluconazole - 8 hr [0.05]
```

Figure 11.

Blastp search of GenBank

Sequences producing significant alignments:　　　　　(bits)　Value

	(bits)	Value
sp|P40030|YEN4_YEAST HYPOTHETICAL 17.1 KD PROTEIN IN SAH1-MEI4 ...	308	1e-83
gnl|PID|e1331605 (AL031854) conserved hypothetical protein [Sch...	110	5e-24
gi|3540193 (AC004122) Unknown protein [Arabidopsis thaliana]	46	1e-04
sp|P54142|SRB7_CAEEL SRB-7 PROTEIN >gi|1584522|prf||2123261V ch...	31	3.4

tblastn search of dbest

Sequences producing significant alignments:　　　　　(bits)　Value

	(bits)	Value
gb|AA271118|AA271118 va86e12.r1 Soares mouse NML Mus musculus c...	81	9e-15
gb|AA048103|AA048103 mj23f09.r1 Soares mouse embryo NbME13.5 14...	81	9e-15
gb|AI172515|AI172515 UI-R-C2p-nu-d-02-0-UI.s1 UI-R-C2p Rattus n...	81	9e-15
gb|AA711847|AA711847 vu59b09.r1 Soares mouse mammary gland NbMM...	81	9e-15
gb|AA153659|AA153659 mq60h05.r1 Soares 2NbMT Mus musculus cDNA ...	80	2e-14
gb|W44146|W44146 mc74h02.r1 Soares mouse embryo NbME13.5 14.5 M...	80	2e-14
gb|AA269958|AA269958 va55c03.r1 Soares mouse 3NME12 5 Mus muscu...	80	2e-14
gb|W08023|W08023 mb37b04.r1 Soares mouse p3NMF19.5 Mus musculus...	78	6e-14
gb|AA014348|AA014348 mi67g10.r1 Soares mouse embryo NbME13.5 14...	73	1e-12
gb|AA272544|AA272544 va75e02.r1 Soares mouse NML Mus musculus c...	73	1e-12
gb|W13627|W13627 ma93h01.r1 Soares mouse p3NMF19.5 Mus musculus...	70	1e-11
gb|W28235|W28235 43h8 Human retina cDNA randomly primed sublibr...	70	2e-11
gb|W27040|W27040 19e6 Human retina cDNA randomly primed sublibr...	68	8e-11

Figure 12.

Mouse EST with similarity to YER044c

```
gb|AI386195|AI386195 mq60h05.y1 Soares 2NbMT Mus musculus cDNA clone
          IMAGE:583161 5' similar to SW:YEN4_YEAST P40030 HYPOTHETICAL
          17.1 KD PROTEIN YER044c. ;, mRNA sequence
          [Mus musculus]
          Length = 455

 Score = 81.5 bits (198), Expect = 6e-15
 Identities = 40/114 (35%), Positives = 68/114 (59%)
 Frame = +3

Query: 23   LPKWLLFISIVSVFNSIQTYVSGLELTRKVYERKPTETTHLSARTFGTWTFISCVIRFYG 82
            L  WL+ +SI+++ N++Q++      L  K+Y  KP     L ARTFG WT +S VIR
Sbjct: 93   LRSWLVMVSIIAMGNTLQSFRDHTFLYEKLYTGKPNLVNGLQARTFGIWTLLSSVIRCLC 272

Query: 83   AMYLNEPHIFELVFMSYMVALFHFGSELLIFRTCKLGKGFMGPLVVSTTSLVWM 136
            A+ ++    ++ +    +++AL HF SEL +F T     G + PL+V++ S++ M
Sbjct: 273  AIDIHNKTLYHITLWTFLLALXHFLSELFVFGTAAPTVGVLAPLMVASFSILGM 434
```

Human EST with similarity to YER044c

```
gb|W28235|W28235 43h8 Human retina cDNA randomly primed sublibrary
          Homo sapiens cDNA.
          Length = 839

 Score = 69.9 bits (168), Expect = 2e-11
 Identities = 33/94 (35%), Positives = 55/94 (58%)
 Frame = +1

Query: 23   LPKWLLFISIVSVFNSIQTYVSGLELTRKVYERKPTETTHLSARTFGTWTFISCVIRFYG 82
            L  WL+ +SI+++ N++Q++      L  K+Y  KP     L ARTFG WT +S VIR
Sbjct: 112  LRSWLVMVSIIAMGNTLQSFRDHTFLYEKLYTGKPNLVNGLQARTFGIWTLLSSVIRCLC 291

Query: 83   AMYLNEPHIFELVFMSYMVALFHFGSELLIFRTC 116
            A+ ++    ++ +    +++AL HF SEL +   C
Sbjct: 292  AIDIHNKTLYHITLWTFLLALGHFLSELFVLWNC 393
```

Figure 13.

Rat EST with similarity to YER044c

```
gb|AI172515|AI172515 UI-R-C2p-nu-d-02-0-UI.s1 UI-R-C2p Rattus
          norvegicus cDNA clone UI-R-C2p-nu-d-02-0-UI 3', mRNA
          sequence [Rattus norvegicus]
          Length = 475

 Score = 80.8 bits (196), Expect = 1e-14
 Identities = 40/114 (35%), Positives = 68/114 (59%)
 Frame = -3

Query: 23   LPKWLLFISIVSVFNSIQTYVSGLELTRKVYERKPTETTHLSARTFGTWTFISCVIRFYG 82
            L  WL+ +SI+++ N++Q++     L  K+Y  KP     L ARTFG WT +S VIR
Sbjct: 404  LRSWLVMVSIIAMGNTLQSFRDHTFLYEKLYTGKPNLVNGLQARTFGIWTLLSSVIRCLC 225

Query: 83   AMYLNEPHIFELVFMSYMVALFHFGSELLIFRTCKLGKGFMGPLVVSTTSLVWM 136
            A+ ++    ++ +    ++++AL HF SEL +F T    G + PL+V++ S++ M
Sbjct: 224  AIDIHNKTLYHITLWTFLLALGHFLSELFVFGTAAPTVGVLAPLMVASFSILGM 63
```

Figure 14.

SUBSTITUTE SHEET (RULE 26)

YLR100w

GenBank No.   1360483

Chromosome   XII

Protein   347 amino acids

                    39,725 Daltons

Comments: unknown function; see S. Huang et al., Biochemistry, 26, pp.
                    8242-46 (1987)

Figure 15.

YLR100w

CYB5

Figure 16.

Treatments Causing Highest Expression of YLR100w

```
Experiment Level      Treatment [baseline]

   6092    8.3        20ug/ml Lovastatin in 1 Ethanol [ABY12.1] - 24 hr [0.15]
   8717    6.7        10ug/ml Simvastatin in 1 DMSO [ABY12.1] - 24 hr [0.14]
   6093    6.3        10ug/ml Lovastatin in 1 Ethanol [ABY12.1] - 24 hr [0.16]
   8716    6.1        7.5ug/ml Simvastatin in 1 DMSO [ABY12.1] - 24 hr [0.13]
   8715    4.9        5ug/ml Simvastatin in 1 DMSO [ABY12.1] - 24 hr [0.12]
   6094    4.4        5ug/ml Lovastatin in 1 Ethanol [ABY12.1] - 24 hr [0.13]
   8705    2.7        [erg11 - ABY210 regulated (100)] - 24 hr [0.17]
   6088    2.6        0.1ug/ml Sulconazole in 1 DMSO [ABY12.1] - 24 hr [0.12]
   8341    2.5        0.025ug/ml Miconazole in 1 DMSO [ABY12.1] - 24 hr [0.15]
   8460    2.4        0.1ug/ml Clotrimazole in 1 DMSO [ABY12.1] - 24 hr [0.12]
   8462    2.3        0.135ug/ml Clotrimazole in 1 DMSO [ABY12.1] - 24 hr [0.17]
   8461    2.3        0.12ug/ml Clotrimazole in 1 DMSO [ABY12.1] - 24 hr [0.14]
   8342    2.3        0.03ug/ml Miconazole in 1 DMSO [ABY12.1] - 24 hr [0.19]
   8703    2.1        [erg11 - ABY210 regulated (80)] - 24 hr [0.14]
   8340    2.0        0.02ug/ml Miconazole in 1 DMSO [ABY12.1] - 24 hr [0.12]
   8463    2.0        0.15ug/ml Clotrimazole in 1 DMSO [ABY12.1] - 24 hr [0.25]
   8701    1.9        [erg11 - ABY210 regulated (60)] - 24 hr [0.14]
```

Figure 17.

Blastp search of GenBank

Sequences producing significant alignments:

	Score (bits)	E Value
pir||S64936 probable membrane protein YLR100w - yeast (Saccharo...	668	0.0
emb|CAA21246| (AL031852) short-chain dehydrogenase [Schizosacch...	183	1e-45
dbj|BAA13878| (D89217) similar to Saccharomyces cerevisiae L800...	182	3e-45
emb|CAA75742| (Y15733) 17-beta-hydroxysteroid dehydrogenase typ...	85	9e-16
gi|1397235 (U44803) ovarian-specific protein [Rattus norvegicus]	84	1e-15
emb|CAB07971| (Z93941) YuxA [Bacillus subtilis] >gi|2635794|emb...	46	5e-04
emb|CAA19277| (AL023705) hypothetical protein [Schizosaccharomy...	43	0.004
dbj|BAA19567| (AB002410) 17-beta-hydroxysteroid dehydrogenase [...	39	0.046
gi|1086892 (U41277) similar to the insect-type alcohol dehydrog...	38	0.079
pir||S56475 hypothetical protein f261a - Escherichia coli >gi|5...	38	0.10
emb|CAA63039| (X91985) glycoprotein 100 [gallid herpesvirus 1]	38	0.10
gb|AAD20218| (AF100931) carbonyl reductase/20beta-hydroxysteroi...	38	0.10

tblastn search of dbest

Sequences producing significant alignments:

	Score (bits)	E Value
gb|AI226514|AI226514 uj07d08.y1 Sugano mouse liver mlia Mus mus...	63	5e-09
gb|AI528381|AI528381 ui96g06.y1 Sugano mouse liver mlia Mus mus...	52	1e-05
gb|R92053|R92053 yp96c01.r1 Homo sapiens cDNA clone 195264 5'.	44	0.003
gb|AI472243|AI472243 tj86g08.x1 Soares_NSF_F8_9W_OT_PA_P_S1 Hom...	37	0.36
gb|AI321571|AI321571 d9f02nm.f1 Neurospora crassa morning cDNA ...	34	3.1
gb|AI211149|AI211149 o0a06a1.r1 Aspergillus nidulans 24hr asexu...	32	9.1
gb|AA219246|AA219246 zq16h06.r1 Stratagene fetal retina 937202 ...	32	9.1

Figure 18.

18/88

Alignment of YLR100w to Mammalian ESTs

gb|AI226514|AI226514 uj07d08.y1 Sugano mouse liver m1ia Mus musculus cDNA
clone
        IMAGE:1891215 5' similar to TR:Q62904 Q62904
        OVARIAN-SPECIFIC PROTEIN. ;, mRNA sequence [Mus
        musculus]   Length = 1039

 Score = 63.2 bits (151), Expect = 5e-09
 Identities = 53/223 (23%), Positives = 108/223 (47%), Gaps = 11/223 (4%)

```
Query: 3    RKVAIVTGTNSNLGLNIVFRLIETEDTNVRLTIVVTSRTLPRVQEVINQIKDFYNKSGRV 62
            RKV ++TG +S +GL + RL+ +D    L + + R L + + V + +  + +
Sbjct: 52   RKVVLITGASSGIGLALCGRLLAEDDD---LHLCLACRNLSKARAVRDTLLASHPSA--- 213

Query: 63   EDLEIDFDYLLVDFTNMVSVLNAYYDINKKYRAINYLFVNAA---------QGIFDGIDW 113
                 +   + +D +++ SV+    ++ +K++ ++YL++NA       + F GI +
Sbjct: 214  -----EVSIVQMDVSSLQSVVRGAEEVKQKFQRLDYLYLNAGILPNPQFNLKAFFCGI-F 375

Query: 114  IGAVKEVFTNPLEAVTNPTYKIQLVGVKSKDDMGLIFQANVFGPYYFISKILPQLTRGK- 172
                V +FT   E +     +   G++        +F+ N+FG + I ++ P L
Sbjct: 376  SRNVIHMFTTA-EGILTQNDSVTADGLQE------VFETNLFGHFILIRELEPLLCHADN 534

Query: 173  -AYIVWISSIMSDPKYLSLNDIELLKTNASYEGSKRLVDLLHLATYKDLKKLGI 225
             + ++W SS +    SL DI+ K    Y  +    DLL++A ++ K G+
Sbjct: 535  PSQLIWTSSRNAKKANFSLEDIQHFKGPEPYSSFQYATDLLNVAXNREFKPEGL 696
```

 gb|AI528381|AI528381 ui96g06.y1 Sugano mouse liver m1ia Mus musculus cDNA
clone
        IMAGE:1890298 5' similar to TR:Q62904 Q62904
        OVARIAN-SPECIFIC PROTEIN. ;, mRNA sequence [Mus
        musculus]   Length = 837

 Score = 52.3 bits (123), Expect = 1e-05
 Identities = 59/260 (22%), Positives = 119/260 (45%), Gaps = 11/260 (4%)

```
Query: 3    RKVAIVTGTNSNLGLNIVFRLIETEDTNVRLTIVVTSRTLPRVQEVINQIKDFYNKSGRV 62
            RKV ++TG +S +GL + RL+ +D    L + + R L + + V + +  + +
Sbjct: 52   RKVVLITGASSGIGLALCGRLLAEDDD---LHLCLACRNLSKARAVRDTLLASHPSA--- 213

Query: 63   EDLEIDFDYLLVDFTNMVSVLNAYYDINKKYRAINYLFVNAA---------QGIFDGIDW 113
                 +   + +D +++ SV+    ++ +K++ ++YL++NA       + F GI +
Sbjct: 214  -----EVSIVQMDVSSLQSVVRGAEEVKQKFQRLDYLYLNAGILPNPQFNLKAFFCGI-F 375

Query: 114  IGAVKEVFTNPLEAVTNPTYKIQLVGVKSKDDMGLIFQANVFGPYYFISKILPQLTRGK- 172
                V +FT   E +     +     + D + +F+ N+   + I ++ P L
Sbjct: 376  SRNVIHMFTTA-EGILTQNDSV------TADRLQEVFETNLSCHFILIRELEPLLLHADN 534

Query: 173  -AYIVWISSIMSDPKYLSLNDIELLKTNASYEGSKRLVDLLHLATYKDLKKLGINQYVVQ 231
             + ++W SS +    SL D +    Y  +    DLL++A + + G+      +
Sbjct: 535  PSQLIWTSSRNAXKANFSLEDXQHSIGPGPYSSFQYATDLLNVALNXNXNQKGLYSSRMC 714

Query: 232  PGIFTSHSFSEYLNFFTYFGMLCLFYLARLL 262
            PG+ ++         TY G+L FYL LL
Sbjct: 715  PGVVMTN--------MTY-GILPPFYLDVLL 780
```

Figure 19.

gb|R92053|R92053 yp96c01.r1 Homo sapiens cDNA clone 195264 5'.Length = 454

 Score = 44.1 bits (102), Expect = 0.003
 Identities = 26/84 (30%), Positives = 40/84 (46%), Gaps = 2/84 (2%)
 Frame = +1

```
Query: 150 FQANVFGPYYFISKILPQLTRGK--AYIVWISSIMSDPKYLSLNDIELLKTNASYEGSKR 207
           F+ NVFG + I ++ P L    + ++W SS +    SL D + K   Y  SK
Sbjct: 1   FETNVFGHFILIRELEPLLCHSDNPSQLIWTSSRSARKSNFSLEDFQHSKGKEPYSSSKY 180

Query: 208 LVDLLHLATYKDLKKLGINQYVVQPG 233
             DLL +A ++  + G+   V  PG
Sbjct: 181 ATDLLSVALNRNFNQQGLYSNVACPG 258
```

Figure 19 (cont).

YER034w

GenBank No.   603267

Chromosome   V

Protein   185 amino acids

                       21,186 Daltons

Comments: unknown function; see S. Huang et al., Biochemistry, 26, pp. 8242-46 (1987)
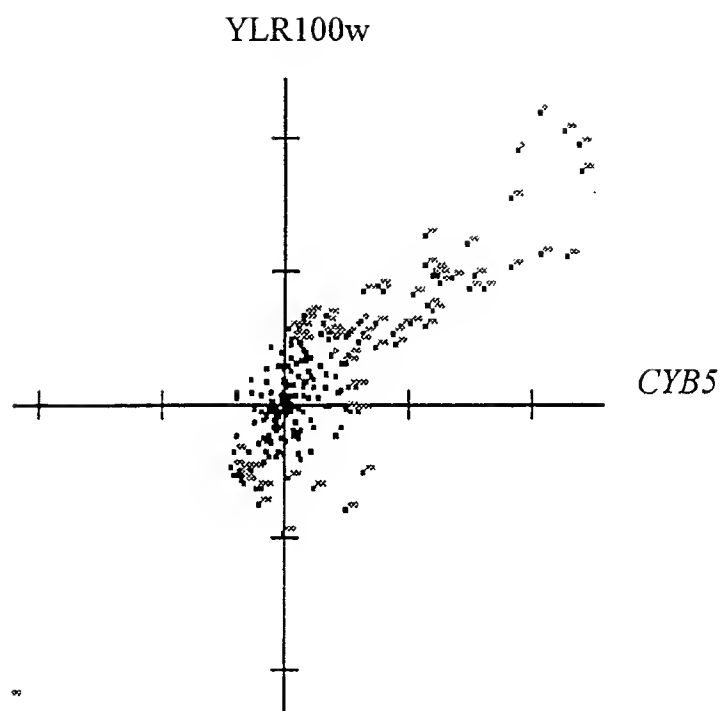
Figure 20

SUBSTITUTE SHEET (RULE 26)

YER034w

GPA2
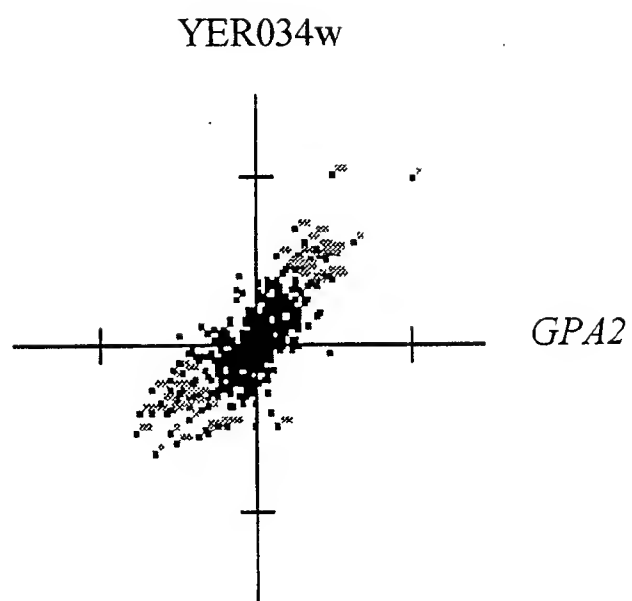
Figure 21.

Mutation of the *YER034w* Gene Leads to Increased Pseudohyphal Growth
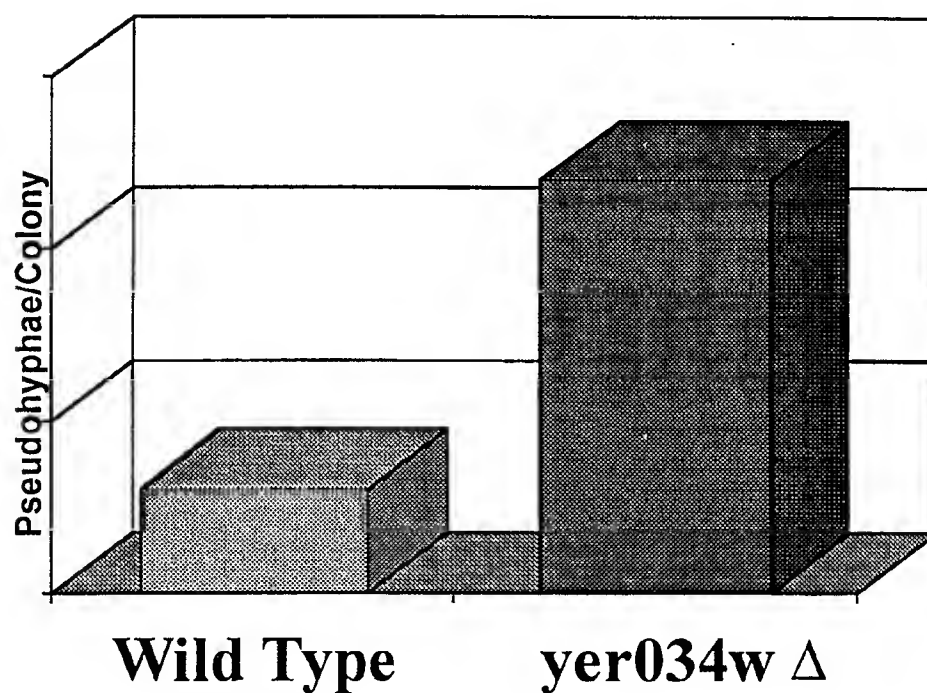
Figure 22.

YKL077w

GenBank No.   486110

Chromosome   XI

Protein   392 amino acids

                     46,042 Daltons

Comments: unknown function; potential transmembrane domain
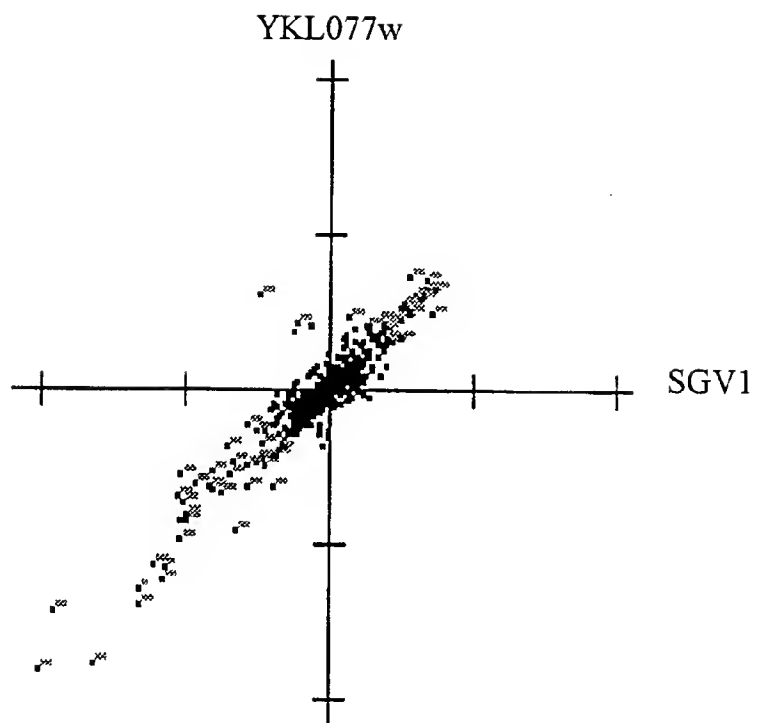
Figure 23

Figure 24.

Expression Correlation of YKL077w

Rank	Gene	Correlation	Exp	Function
1	YKL077w	+1.00	0.5 - 9.1	
2	SGV1	+0.92	0.7 - 14.4	CDC28/cdc2 related protein kinase
3	RHO1	+0.88	1.3 - 20.9	GTP-binding protein
4	YKL075c	+0.86	0.2 - 2.5	
5	SRA3	+0.84	0.3 - 4.6	catalytic subunit of PKA
6	RPB4	+0.84	0.3 - 7.8	subunit of RNA polymerase II
7	PKC1	+0.84	0.6 - 11.7	putative protein kinase

Figure 25.

Blastp search of GenBank

Sequences producing significant alignments:

	Score (bits)	E Value
sp|P36081|YKH7_YEAST HYPOTHETICAL 46.0 KD PROTEIN IN SMY1-MUD2 ...	785	0.0
gi|1172087 (U19568) squamous cell carcinoma antigen [Homo sapie...	35	0.75
sp|P54634|POLN_LORDV NON-STRUCTURAL POLYPROTEIN [CONTAINS: RNA-...	35	0.75
sp|P29508|SCC1_HUMAN SQUAMOUS CELL CARCINOMA ANTIGEN 1 (SCCA-1)...	35	0.75
pir||I38201 squamous cell carcinoma antigen 1 - human	35	0.75
gi|3063469 (AC003981) F22013.31 [Arabidopsis thaliana]	35	0.98
pir||S23760 polyphenolic adhesive protein - blue mussel (fragme...	34	2.2
sp|P37222|MAOC_LYCES MALATE OXIDOREDUCTASE, CHLOROPLAST (MALIC ...	32	5.0

tblastn search of dbest

Sequences producing significant alignments:

	Score (bits)	E Value
gb|T38483|T38483 EST103979 Saccharomyces cerevisiae cDNA 3' end.	99	6e-20
gb|T36426|T36426 EST101359 Saccharomyces cerevisiae cDNA 3' end.	69	1e-10
gb|AA724136|AA724136 ai07b06.s1 Soares parathyroid tumor NbHPA ...	32	9.2

Figure 26.

YGR046w

GenBank No.   1323049

Chromosome   VII

Protein   385 amino acids

                     44,219 Daltons

Comments: essential gene in yeast

Figure 27
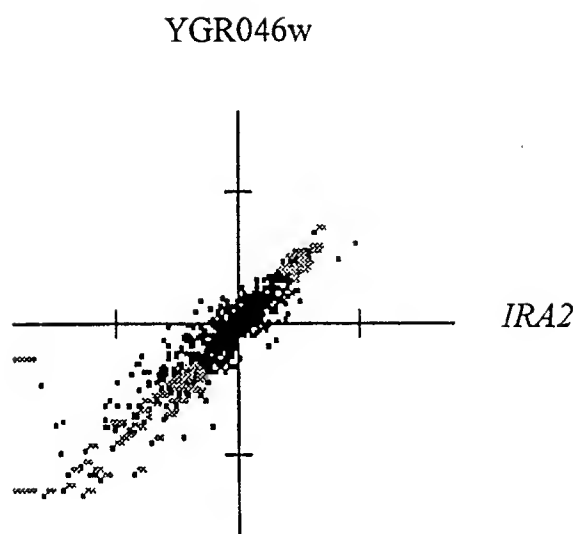
YGR046w

IRA2

Figure 28.

Expression Correlation to YGR046w

Gene	Correlation	Levels	Function
YGR046w	+1.00	0.9 - 10.1	similar to phage 1C ANTP-139 protein PIR:S46430
IRA2	+0.90	0.3 - 5.4	GTPase activating protein, neurofibromin homolog
RLR1	+0.89	0.8 - 6.1	Regulatory protein, post-transcription initiation
NUT1	+0.85	0.4 - 3.2	Negative regulator of HO endonuclease promoter
SRO7	+0.84	0.3 - 4.9	Drosphila tumor suppressor homolog, rho3 suppressor
DST1	+0.84	0.5 - 4.5	RNA polymerase II elongation factor
MTR3	+0.84	1.4 - 11.8	mRNA transport
TPD3	+0.82	2.6 - 22.0	protein phosphatase (PP2A regulatory subunit)
SYF3	+0.80	0.1 - 2.0	similar to Drosophila probable cell cycle control
MEX67	+0.78	1.2 - 14.1	INvolved in nuclear mRNA export, binds both poly(A)
YNK1	+0.78	1.1 - 24.2	Nucleoside diphosphate kinase
MPD2	+0.78	0.3 - 6.0	protein disulfide isomerase related protein
BEM2	+0.77	0.7 - 10.7	Rho-type GTPase activating protein (GAP

Figure 29.

Treatments Causing the Most Significant Changes in Expression of YGR046w

Experiment	Levels	Log ratio	Treatment [baselines]
11757	10.1/5.0	0.9	20ug/ml 2,4-Dinitrophenol in 1% DMSO [ABY12 6144C yx-101] - 24 hr [0.19/0.05]
7571	7.3/3.9	0.8	2000ug/ml p-Aminosalicylic Acid in 2% DMSO [ABY12.1] - 24 hr [0.21/0.08]
10815	7.7/4.3	0.8	1500ug/ml Acetylsalicylic Acid in 1% DMSO [ABY12] - 24 hr [0.20/0.05]
10482	8.2/4.6	0.7	600ug/ml Sodium Nitrite in 1% DMSO (ABY12.1] - 24 hr [0.23/0.07]
10819	7.2/4.2	0.7	900ug/ml Acetylsalicylic Acid 10 ug/ml Methotrexate in 1% DMSO [ABY12] - 24 hr [0.14/0.05]
7877	8.2/4.8	0.7	1200ug/ml p-Aminosalicylic Acid in 1% DMSO [ABY12.1] - 24 hr [0.22/0.09]
10814	7.3/4.3	0.7	1200ug/ml Acetylsalicylic Acid in 1% DMSO [ABY12] - 24 hr [0.15/0.05]
10822	7.0/4.2	0.7	300ug/ml Acetylsalicylic Acid 20 ug/ml Methotrexate in 1% DMSO [ABY12] - 24 hr [0.09/0.05]
9107	6.3/3.8	0.7	550ug/ml Thiourea in 1% DMSO [ABY12.1] - 24 hr [0.12/0.05]
7573	6 5/3.9	0.7	2130ug/ml p-Aminosalicylic Acid in 2% DMSO [ABY12.1] - 24 hr [0.21/0.08]
10481	7.6/4.6	0.7	500ug/ml Sodium Nitrite in 1% DMSO [ABY12.1) - 24 hr [0.18/0.07]
8362	5.6/3.4	0.7	6ug/ml 8-Hydroxyquinoline in 1% DMSO [ABY12.1] - 24 hr [0.07/0.06]
9613	6.2/3.8	0.7	0.1ug/ml Azoxystrobin in 1% DMSO [ABY12.1] - 24 hr [0.15/0.06]

Figure 30.

Experiment	Levels	Log ratio	Treatment [baselines]
10983	2.2/5.2	-0.7	50ug/ml Maleimide in 1% DMSO [ABY12] – 24 hr [0.14/0.07]
8737	2 0/5.0	-0.7	2ug/ml 5-Fluorocytosine in 1% DMSO [ABY12.1] – 24 hr (0.12/0.06)
8263	1.8/4.4	-0.7	4.5ug/ml Dimethyl Sulfoxide in 1% DMSO [ABY12.1] – 24 hr [0.18/0.6]
11679	1.6/4.0	-0.7	600ug/ml Tricyclazole in 2% DMSO [ABY 12 6144C yx-101] – 24 hr (0.33/0.05]
8435	1.8/4.6	-0.8	20ug/ml Benomyl in 1% DMSO [ABY12.1] – 24 hr [027/0.06]
10802	1.6/4 2	-0.8	20ug/ml Cumene Hydroperoxide in 1% DMSO [ABY12] – 24 hr [0.11/0.06]
9633	1.4/3.7	-0.8	80ug/ml Pyrimethanil in 1% DMSO [ABY12.1] – 24 hr [0.27/0.06]
9340	1.3/4.0	-0.9	0.03ug/ml Cycloheximide in 1% DMSO [ABY12.1] – 24 hr [0.25/0.06]
8354	-1.3/4.1	-1	100ug/ml Quinacrine in 1% DMSO (ABY12.1] – 24 hr [0.10/0.06]
11774	1.5/4.7	-1	5ug/ml Sodium azide in 1% DMSO [ABY 12 6144C yx-101] – 24 hr [0.13/0.06]
9588	1.1/3.5	-1	600ug/ml Hydrogen Peroxide in 1% DMSO [ABY12.1] – 24 hr [0.14/0.08]
10574	1.7/5.3	-1	300ug/ml Pyroquilon in 1% DMSO [ABY12] – 24 hr [0.34/0.10]
12366	1.4/4.7	-1	0.2ug/ml Thimerosal in 1% DMSO [ABY 12 6144C yx-101] – 24 hr [0.10/0.07]
8429	1.1/3.8	-1.1	250ug/ml Benfluorex Hydrochloride in 1% DMSO [ABY12.1] – 24 hr [0.15/0.06]
11775	1.3/4.7	-1.1	6ug/ml Sodium azide in 1% DMSO [ABY 12 6144C yx-101] – 24 hr [0.21/0.06]
12327	1.4/5.1	-1.1	1000ug/ml Benzimidazole in 1% DMSO [ABY12 6144C yx-101] – 24 hr [0.16/0.07]
11595	0.9/4.7	-1.5	10ug/ml Sodium azide in 1% DMSO [ABY 12 6144C yx-101] – 24 hr [0.27/0.05]

Figure 30 (cont).

YJR041c

GenBank No.   1015693

Chromosome   X

Protein   1173 amino acids

                     135,096 Daltons

Comments: essential gene in yeast; contains a leucine zipper; potential
                     transmembrane domain

Figure 31
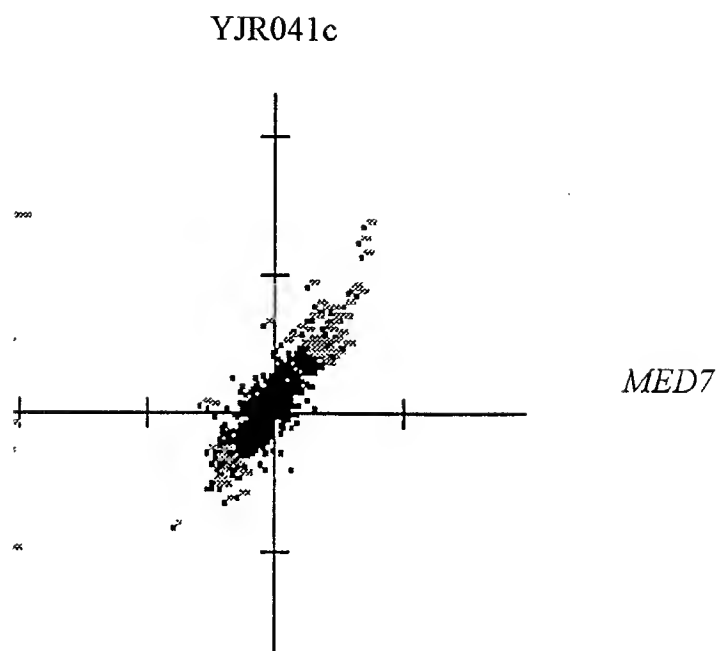
YJR041c

MED7

Figure 32.

Correlation to YJR041c

Gene	Correlation	Exp	Function
YJR041c	+1.00	0.2 - 1.7	similar to Podospora anserina NADH dehydrogenase
MED7	+0.83	0.3 - 0.9	Stoichiometric member of mediator complex
SNP2	+0.82	0.5 - 2.0	snRNP G protein (the homologue of the human Sm-G)
RPA43	+0.82	0.5 - 2.4	DNA-dependent RNA polymerase I subunit A43
SLS1	+0.81	0.3 - 1.5	73 kDa mitochondrial integral membrane protein
SEC53	+0.80	0.7 - 4.1	phosphomannomutase
EMP47	+0.76	0.3 - 1.5	47 kDa type I transmembrane protein
POP4	+0.76	0.2 - 1.0	RNase P and RNase MRP subunit
RPL1A	+0.75	1.4 - 5.8	homolog of bacterial ribosomal proteins of L1 family
RPC53	+0.74	0.2 - 0.9	RNA polymerase III (C) subunit
SKI6	+0.72	0.5 - 2.4	ExtraCellular Mutant; ribosomal RNA processing
UPF3	+0.72	0.1 - 0.4	Stimulates decay of mRNAs with premature stop codons
TAF19	+0.71	0.1 - 0.4	TFIID subunit
RPL37B	+0.71	1.5 - 7.8	60S ribosomal protein YL35
GCD10	+0.71	0.5 - 2.3	RNA-binding subunit, translation initiation factor 3

Figure 33.

Blastp search of GenBank

Sequences producing significant alignments:

	Score (bits)	E Value
sp|P47108|YJl1_YEAST HYPOTHETICAL 135.1 KD PROTEIN IN GEF1-NUP8...	2238	0.0
emb|CAA89570| (Z49542) ORF YJR041c [Saccaromyces cerevisiae]	2127	0.0
sp|Q09804|YAB2_SCHPO HYPOTHETICAL 150.5 KD PROTEIN C2G11.02 IN ...	53	8e-06
emb|CAA91167| (Z54354) hypothetical protein [Schizosaccharomyce...	53	8e-06
gi|3929312 (AF100426) fimbriae-associated protein Fap1 [Strepto...	43	0.012
gi|2688777 (AE001181) exonuclease SbcC (sbcC) [Borrelia burgdor...	38	0.51
gi|2462828 (AF000657) hypothetical protein [Arabidopsis thaliana]	35	2.6
pir||S43557 coiled coil protein B0284.1 - Caenorhabditis elegan...	35	2.6
gi|2315501 (AF016451) No definition line found [Caenorhabditis ...	35	2.6
sp|P13496|DYNA_DROME 150 KD DYNEIN-ASSOCIATED POLYPEPTIDE (DP-1...	34	5.8
sp|Q58042|Y625_METJA HYPOTHETICAL ATP-BINDING PROTEIN MJ0625 >g...	34	5.8
gb|AAD18581| (AE001626) ClpC Protease [Chlamydia pneumoniae]	34	5.8
gi|3098583 (AF049495) gag polyprotein [Human immunodeficiency v...	34	7.5
sp|P44581|NHAA_HAEIN NA(+)/H(+) ANTIPORTER 1 >gi|1075053|pir||C...	33	9.9
gi|2062752 (U92845) kinesin motor protein [Ustilago maydis]	33	9.9

tblastn search of dbest

Sequences producing significant alignments:

	Score (bits)	E Value
gb|AI201151|AI201151 qf64h07.x1 Soares_testis_NHT Homo sapiens ...	36	3.7
gb|AA747649|AA747649 nx77g11.s1 NCI_CGAP_Ew1 Homo sapiens cDNA ...	34	8.4
gb|AI248270|AI248270 qh75g09.x1 Soares_fetal_liver_spleen_1NFLS...	34	8.4

Figure 34.

HES1

GenBank No.   1420543

Chromosome   XV

Protein   433 amino acids

                     49,502 Daltons

Comments: implicated in ergosterol pathways; related to human oxysterol
                     binding protein

Figure 35

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Expression Correlation to HES1

Gene	Correlation	Exp	Function
HES1	+1.00	0.1 - 7.2	homology to human oxysterol binding protein
ERG2	+0.90	0.1 - 5.3	C-8 sterol isomerase
PAU5	+0.89	0.1 - 4.7	member of seripauperin protein/gene family
ERG7	+0.83	0.2 - 3.0	lanosterol synthase
CYB5	+0.83	0.4 - 17.8	cytochrome b5
YJL105w	+0.81	0.1 - 4.7	similar to Ykr029p
YER044c	+0.79	0.3 - 3.7	
ERG11	+0.79	0.3 - 13.0	cytochrome P450 lanosterol 14a-demethylase
HEM14	+0.76	0.1 - 1.3	protoporphyrinogen oxidase
ERG9	+0.76	0.8 - 8.8	squalene synthetase
TIR1	+0.74	0.2 - 6.8	cold-shock induced - serine-alanine-rich
ERG8	+0.70	0.3 - 6.0	phosphomevalonate kinase
ERG6	+0.69	0.5 - 9.6	SAM: delta 24-methyltransferase

Figure 36.

Treatments that Induce the *HES1* Reporter

Experiment	Levels	Log ratio	Treatment [baselines]
9923	7.2/0.1	4.1	20ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.19/0.07]
9930	4.6/0.1	3.6	40ug/ml Lovastatin in 1% DMSO [ABY12.1] - 24 hr [0.18/0.07]
9708	4.5/0.1	3.6	15ug/ml Atorvastatin in 1% DMSO [ABY12.1] - 24 hr [0.17/0.05]
9709	4.4/0.1	3.6	20ug/ml Atorvastatin in 1% DMSO [ABY12.1] - 24 hr [0.16/0.05]
9924	4.2/0.1	3.6	40ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.27/0.07]
6092	4.4/0.1	3.5	20ug/ml Lovastatin in 1% Ethanol [ABY12.1] - 24 hr [0.16/0.08]
9909	3.8/0.1	3.5	10ug/ml Fluvastatin in 1% DMSO [ABY12.1] - 24 hr [0.18/0.07]
9707	3.7/0.1	3.5	10ug/ml Atorvastatin in 1% DMSO [ABY12.1] - 24 hr [0.15/0.05]
8465	3.0/0.1	3.2	0.03ug/ml Econazole in 1% DMSO [ABY12.1] - 24 hr [0.26/0.06]
9922	2.8/0.1	3.2	10ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.14/0.07]
8463	2.7/0.1	3.1	0.15ug/ml Clotrimazole in 1% DMSO [ABY12.1] - 24 hr [0.25/0.06]
9797	2.6/0.1	3.1	6ug/ml Fluvastatin in 1% DMSO [ABY12.1] - 24 hr [0.12/0.05]
6093	2.7/0.1	3	10ug/ml Lovastatin in 1% Ethanol [ABY12.1] - 24 hr [0.18/0.08]
8717	2.4/0.1	3	10ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.14/0.06]

Figure 37.

Experiment	Levels	Log ratio	Treatment [baselines]
9706	2.2/0.1	2.9	5ug/ml Atorvastatin in 1% DMSO [ABY12.1] - 24 hr [0.11/0.05]
9908	2.1/0.1	2.9	6ug/ml Fluvastatin in 1% DMSO [ABY12.1] - 24 hr [0.14/0.07]
9929	2.1/0.1	2.8	20ug/ml Lovastatin in 1% DMSO [ABY12.1] - 24 hr [0.16/0.07]
8716	2.1/0.1	2.8	7.5ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.13/0.06]
8461	1.8/0.1	2.7	0.12ug/ml Clotrimazole in 1% DMSO [ABY12.1] - 24 hr [0.14/0.06]
8342	1.8/0.1	2.7	0.03ug/ml Miconazole in 1% DMSO [ABY12.1] - 24 hr [0.19/0.06]
9796	1.7/0.1	2.7	4ug/ml Fluvastatin in 1% DMSO [ABY12.1] - 24 hr [0.10/0.05]
8462	1.7/0.1	2.7	0.135ug/ml Clotrimazole in 1% DMSO [ABY12.1] - 24 hr [0.17/0.06]
6088	1.4/0.1	2.6	0.1 ug/ml Sulconazole in 1% DMSO [ABY12.1] - 24 hr [0.12/0.07]
8341	1.5/0.1	2.5	0.025ug/ml Miconazole in 1% DMSO [ABY12.1] - 24 hr [0.15/0.06]
8460	1.3/0.1	2.4	0.1ug/ml Clotrimazole in 1% DMSO [ABY12.1] - 24 hr [0.12/0.06]
8715	1.3/0.1	2.3	5ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.12/0.06]
9921	1.1/0.1	2.3	5ug/ml Simvastatin in 1% DMSO [ABY12.1] - 24 hr [0.18/0.07]

Figure 37 (cont).
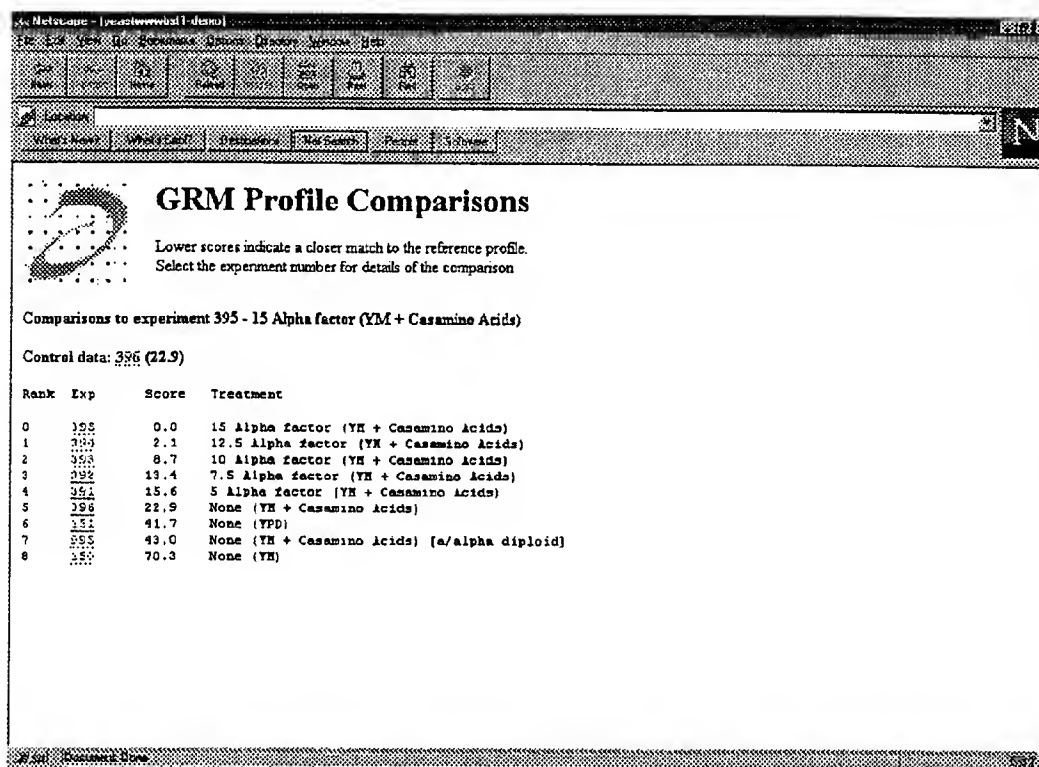
Figure 38

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FIGURE 39.   *YJL105w* DNA Sequence

Sequence contains 1200bp of 5' promoter sequence.
Symbols: 1 to: 2883   from: chr10.gcg   ck: 4711,
223552 to: 226434
Chromosome X Sequence
EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide
sequence of Saccharomyces cerevisiae chromosome X.
Galibert, F., Alexandraki, D., Baur, A., Boles, E.,
Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De
Haan, M., Domdey, H., . . .

gcgseq.tmp.4454 Length: 2883 March 26, 1999 16:51 Type:
N Check: 6274   ..

```
   1   TGGAAAAGCT  CACTGTGAGG  TTCCTTGGAG  CCAATAGTAA  TACAGCACAA
  51   TCCAAGGAAA  AATCTGGCCT  ATATGCAAGG  AAGGAGAGAT  AGTCAAAAGC
 101   ATTCTTTCCC  CTAGAAGTTG  GTGCATATAT  GGCATCGTTA  AAACATATTA
 151   CCCCCAAAAT  TTCTTCTCTA  AACGATGTGC  TTGGCCTTTG  TTTTGGTTTT
 201   TGATGTCGGT  CGTTTGAGGC  CCCTTGCGGA  AAATCGAGAT  CGCCGAATGG
 251   CACGCGAGGG  AAGGGAAATA  AGGTTTAAAG  GCACTGAAAC  AATAGGCAAG
 301   AAGTAGGCGA  GAGCCGACAT  ACGAGACTAA  TGTGTCCGCG  TTTCTAAGGC
 351   CACTTTTCAA  TGAAACGGAT  ATTGATATGC  TAGTAAAAGG  ACGAGCTCAA
 401   GAGCGAAAAT  ATAAGTAAAG  AATTCGAGTG  CACTTGTCTC  CATGCAGCAA
 451   GATTTCATAT  GAGTCTTTTT  TATCTTTTTA  CTTTTTACAT  TACACGATAT
 501   GCACTTTATG  AAAATTTAAC  GAGGTTGGAA  GCCGGATAAT  CAACCAAAAT
 551   CAGGCACGAA  GGCACACTCG  TATATGCATG  TTGTTGAAAC  TCTGTTACGC
 601   TGAACTAACA  ATCACACATG  TAGAGGTCAC  CGGGAAAAGT  TGCGACCCCA
 651   TGGAAGGTCG  ATCTCTTCGT  TTGGCTTTGC  TTGGCTGGCG  GCATTGCGCT
 701   TCTTCGCTTA  TACCCGTCTC  TTGACGCTCG  AGCTCGTTCA  TTGAGATACC
 751   TTTATTCTTG  CACATTTTCT  GGCTTTTTTC  GCTACTCGGG  TACATGTAAT
 801   CATGCACACA  GAAGGTGCTG  TAGGGTGAAA  GTTCCTTTGT  GCTGTCGTTT
 851   GTTTTTAATG  CCAAACTTTC  TGGTGATCAA  TAACCACCTC  TTTTTCCTTC
 901   AGGAAACCTT  ATTATTGTTC  TTGGATAGTA  CTAGGAAGTA  TATAAGGAAC
 951   CTCGATTTTG  GTATTGCACG  GCTATACACA  TCTAAGAAAC  TTTGTATAAA
1001   AGGTGGCTAC  CCTATTCATA  GCTTGATATC  AATAGGCCAT  CTCATCACTT
1051   TTTATTGAAA  AGGAAGGAG   GGAAATATAT  CTGATTCAAA  TTACTTGTTT
1101   GCTTCTCTTT  AAGACAAAAG  CATAGATAAT  TTCAGCGTGG  AACGCCGGAA
1151   TAAGATTGGT  ACCCTCGTCA  GAAAGTTACA  AATACCGCTT  CATCTTCAAA
1201   ATGACTTCAC  CGGAATCACT  ATCTTCTCGT  CATATCAGGC  AAGGAAGGAC
1251   ATACACAACC  ACAGACAAGG  TCATATCGCG  GTCGTCGTCG  TACTCATCTA
1301   ATAGTTCAAT  GTCTAAAGAT  TACGGCGATC  ACACACCCTT  GTCCGTCAGC
1351   AGTGCAGCTT  CAGAGACATT  ACCCTCACCT  CAGTATATGC  CGATAAGGAC
1401   ATTCAATACA  ATGCCTACAG  CTGGCCCAAC  GCCTTTACAT  TTATTTCAAA
1451   ATGACAGGGG  CATTTTCAAC  CATCATTCTT  CATCAGGCTC  ATCAAAAACG
1501   GCATCAACAA  ATAAAGAGG   AATAGCAGCA  GCAGTAGCAT  TGGCAACTGC
1551   TGCCACCATA  CCATTTCCAC  TGAAAAAACA  GAATCAAGAT  GATAATTCCA
1601   AGGTCTCGGT  AACACACAAT  GAATCATCGA  AAGAAATAA   AATTACACCC
1651   TCCATGAGAG  CAGAAGATAA  CAAACCTAAA  AATGGTTGCA  TCTGCGGTTC
1701   AAGTGACTCC  AAGGATGAGT  TGTTTATACA  GTGTAACAAA  TGTAAACGT
1751   GGCAGCACAA  GTTATGTTAT  GCTTTCAAAA  AATCAGATCC  AATAAAAAGA
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SUBSTITUTE SHEET (RULE 26)

```
1801  GATTTTGTTT GCAAAAGATG TGACAGTGAT ACGAAAGTGC AGGTTAATCA
1851  AGTAAAACCA ATGATATTCC CTAGAAAAAT GGGAGATGAG CGATTATTTC
1901  AATTTTCATC CATAGTGACA ACTTCAGCAT CGAACACAAA TCAGCATCAA
1951  CAGTCTGTGA ATAACATAGA GGAACAGCCC AAGAAACGTC AACTTCATTA
2001  TACCGCCCCA ACAACTGAAA ATAGCAATAG TATACGGAAA AAATTGAGGC
2051  AAGAAAAACT GGTAGTATCA AGCCACTTTC TGAAGCCACT ACTGAATGAG
2101  GTAAGTTCTT CCAATGACAC GGAATTCAAA GCAATAACAA TATCAGAGTA
2151  TAAGGACAAA TATGTTAAGA TGTTTATTGA TAACCATTAT GATGACGATT
2201  GGGTTGTTTG TTCTAACTGG GAAAGCTCAA GGTCAGCTGA CATCGAGGTA
2251  AGAAAATCAT CAAATGAAAG AGATTTTGGA GTCTTCGCTG CAGATTCTTG
2301  TGTTAAAGGT GAGCTAATTC AAGAATATTT GGGCAAAATT GATTTTCAAA
2351  AAAATTATCA GACAGATCCA AATAATGACT ATCGTTTGAT GGGAACGACA
2401  AAACCTAAAG TACTTTTTCA TCCACATTGG CCTTTATATA TAGACTCTCG
2451  AGAAACAGGC GGATTAACAA GATACATAAG ACGGAGTTGT GAGCCCAATG
2501  TGGAACTAGT AACGGTAAGA CCGCTTGACG AAAAACCAAG AGGAGATAAT
2551  GATTGTAGAG TTAAATTTGT TTTAAGGGCT ATAAGAGATA TTCGTAAGGG
2601  AGAAGAGATA AGCGTAGAAT GGCAATGGGA TTTGAGAAAT CCTATTTGGG
2651  AGATAATAAA TGCATCTAAA GATTTGGATT CCCTACCGGA TCCCGACAAG
2701  TTCTGGTTGA TGGGGTCAAT AAAGACTATT TTAACAAATT GTGATTGTGC
2751  ATGTGGGTAC TTGGGCCATA ATTGTCCAAT AACTAAAATC AAAAACTTTT
2801  CTGAAGAATT CATGAGGAAT ACGAAGGAAT CCCTATCTAA TAAATCTTAC
2851  TTTAATACAA TAATGCACAA CTGTAAGCCA TAA
```

FIGURE 39 (cont).

FIGURE 40. *YJL105W* Protein Sequence

EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of
Saccharomyces cerevisiae chromosome X.

Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N.,
Chuat, J. C., Coster, F., Cziepluch, C., De Haan, M., Domdey, H.,
Durand, P., Entian, K. D., Gatius, M., Goffeau, A., Grivell, L. A., et al.

YJL105W Length: 560 March 26, 1999 16:52 Type: P Check: 103 ..

```
    1  MTSPESLSSR HIRQGRTYTT TDKVISRSSS YSSNSSMSKD YGDHTPLSVS

   51  SAASETLPSP QYMPIRTFNT MPTAGPTPLH LFQNDRGIFN HHSSSGSSKT

  101  ASTNKRGIAA AVALATAATI PFPLKKQNQD DNSKVSVTHN ESSKENKITP

  151  SMRAEDNKPK NGCICGSSDS KDELFIQCNK CKTWQHKLCY AFKKSDPIKR

  201  DFVCKRCDSD TKVQVNQVKP MIFPRKMGDE RLFQFSSIVT TSASNTNQHQ

  251  QSVNNIEEQP KKRQLHYTAP TTENSNSIRK KLRQEKLVVS SHFLKPLLNE

  301  VSSSNDTEFK AITISEYKDK YVKMFIDNHY DDDWVVCSNW ESSRSADIEV

  351  RKSSNERDFG VFAADSCVKG ELIQEYLGKI DFQKNYQTDP NNDYRLMGTT

  401  KPKVLFHPHW PLYIDSRETG GLTRYIRRSC EPNVELVTVR PLDEKPRGDN

  451  DCRVKFVLRA IRDIRKGEEI SVEWQWDLRN PIWEIINASK DLDSLPDPDK

  501  FWLMGSIKTI LTNCDCACGY LGHNCPITKI KNFSEEFMRN TKESLSNKSY

  551  FNTIMHNCKP
```

FIGURE 41. *YMR134w* **DNA Sequence**

Sequence contains 1200bp of 5' promoter sequence.
Symbols: 1 to: 1914 from: chr13.gcg   ck: 8335, 536637 to: 538550
Chromosome XIII Sequence
Nature 387:90-93 [97313268] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XIII.
Bowman, S., Churcher, C., Badcock, K., Brown, D., Chillingworth, T.,
Connor, R., Dedman, K., Devlin, K., Gentles, S., Hamlin, N., Hunt, S., . . .

gcgseq.tmp.31828 Length: 1914 March 26, 1999 16:58 Type: N Check: 3324
..

```
   1    TACAATAACA AGCCAGGTGC AAGGCAATAA TAACGGTACA AAGGTCTGTT
  51    TCACAGAAGG TCCAAAAGTT AGTAGCTACA CAAATCCGAA CACGCAATTT
 101    CAAACTCAAA ACATGATTAT GGATTTCAGT CAACGTTATC AGGAAGAATC
 151    TGAAAGAGAG TCAAATAATC GTTCAAATAT AACTTTACCA CACGACAGCA
 201    TTCAAATAGC TCAACAAATA TGGCCAAACA CGGATTTAAA TGTAGTACAA
 251    TCTTCACAAG ACCTCAACAC TCCAATGGCT ACGCAAACTG TTTTGGGTCG
 301    TCCTGAGTCG CTAATTGTAC AGCCATTGGA GGTTTCTCAA TCTCCACCAA
 351    ACACTACCAA CTGCCTTCCT AATGCAGAAA ACAAAAAGAA AAAAGTCGAC
 401    ACCACTTCTG ATTTTACTTC AAGAAAGGAG ATTGCTCTGT GTAAAACTGG
 451    TTTATTAGAA ACTATTCATA TACCAAAGGA AAGGGAAAGT CAGATGCAAA
 501    GCGTCACTGG TTTAGATGCA ACACCAACGA TTATATGGAG CCCCGGGAAA
 551    GACAACACGG CGAAGAAAAA TACCAGTAAT AAGAAAAATA TTGATGATAA
 601    ACTAACAAAC CCCCAAAAAT CTGGAAATAC ACATACCCCT GATAGAAATA
 651    AAGAAGTGCT ACCTAACGGC ACACTTAATG AAACGAGGAA AGAAGCATCG
 701    CCAAGCGAAG GATTAACGAT AAGAGTTAAA AACGTTAATC GGAATGCGTC
 751    AAGAAAAATA TCTAAGCGGC TAATCAAGGA AAAGTTGAAA GACGAAGAAT
 801    TCATGAAATG GGTATGTATG CATTTGCAAG AAACTGAGCT GTTTCCCCCT
 851    CTTATCCACT CATTTTCTCT GACTTGACAA AGAAATACTA ACTAACAACT
 901    TTTGCCACTA CAAATATGAA TGAAAAGGTT AATAAGGTTG AAACGGTTCT
 951    CAATAAAATG TTCGAAAAGT GAACCCTTTT TTTGCAATTC CTTTTTACAC
1001    TAGCCACGAA GTAAATGGA AAAGTAAACC CGAGTTTCGG CAATATCGCT
1051    AAGCAAGAAG AGCAAGCTCG TTTAAGTAAG CCTTTATGAA AAAAAAACAA
1101    AATATAAAGC ATTATAAAAA TTGAATCACA TCGCAAATCT GCAATATACT
1151    TGGAAGTGTT TATAGCAAAG TGTGGTATAG AAAAAGAACC AAAGGCCGGT
1201    ATGTCGTTAA AGGATAGGTA TCTAAATCTC GAATTAAAAT TAATAAATAA
1251    ACTACAGGAG TTGCCATATG TTCATCAATT TATCCATGAT CGAATAAGTG
1301    GTAGGATAAC TCTCTTTTTG ATAGTGGTTG GTACGCTTGC ATTTTTTAAC
1351    GAACTGTATA TAACGATCGA AATGAGTCTT CTACAAAAGA ACACATCAGA
1401    AGAACTAGAG CGTGGAAGAA TCGATGAAAG TCTGAAGCTT CATCGGATGT
1451    TGGTGAGTGA TGAATATCAC GGTAAAGAAT ACAAAGACGA GAAAAGCGGT
1501    ATTGTTATTG AAGAGTTCGA AGATCGCGAT AAGTTTTTTG CAAAACCTGT
1551    GTTTGTATCA GAATTGGATG TCGAATGTAA TGTTATTGTA GATGGGAAAG
1601    AACTTCTGTC CACCCCATTA AAATTTCATG TTGAATTTTC TCCAGAGGAT
1651    TATGAAAATG AAAAAAGACC TGAGTTTGGT ACTACCTTGC GTGTATTGAG
1701    GCTGAGACTT TACCACTACT TTAAAGATTG CGAAATATAT CGCGATATAA
1751    TTAAGAATGA GGGCGGTGAA GGGGCAAGAA AGTTTACGAT TTCCAACGGT
1801    GTCAAAATTT ACAATCATAA AGATGAACTA CTGCCATTGA ATATCGATGA
1851    TGTTCAATTA TGTTTCCTGA AGATTGATAC GGGAAACACG ATAAAATGCG
1901    AATTCATACT ATGA
```

FIGURE 42. *YMR134w* **Protein Sequence**

Nature 387:90-93 [97313268] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XIII.

Bowman, S., Churcher, C., Badcock, K., Brown, D., Chillingworth, T.,
Connor, R., Dedman, K., Devlin, K., Gentles, S., Hamlin, N., Hunt, S.,
Jagels, K., Lye, G., Moule, S., Odell, C., Pearson, D., Rajandream, et al.

  YMR134W Length: 237 March 26, 1999 16:59 Type: P Check: 2966 ..

```
    1  MSLKDRYLNL ELKLINKLQE LPYVHQFIHD RISGRITLFL IVVGTLAFFN

   51  ELYITIEMSL LQKNTSEELE RGRIDESLKL HRMLVSDEYH GKEYKDEKSG

  101  IVIEEFEDRD KFFAKPVFVS ELDVECNVIV DGKELLSTPL KFHVEFSPED

  151  YENEKRPEFG TTLRVLRLRL YHYFKDCEIY RDIIKNEGGE GARKFTISNG

  201  VKIYNHKDEL LPLNIDDVQL CFLKIDTGNT IKCEFIL
```

FIGURE 43. *YER044c* DNA Sequence

Sequence contains 1200bp of 5' promoter sequence.

Symbols: 1 to: 1647 from: chr5.gcg /rev   ck: 9036, 237569 to: 239215

Chromosome V Sequence
Nature 387:78-81 [97313264] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome V.
Dietrich, F. S., Mulligan, J., Hennessy, K., Yelton, M. A., Allen, E.,
Araujo, R., Aviles, E., Berno, A., Brennan, T., Carpenter, J., Chen, . . .

gcgseq.tmp.2512 Length: 1647 March 26, 1999 16:38 Type: N Check: 8794 ..

```
   1  AACACTCCAA ATCTTGTTAG TTTCTCATTA TTCGCATCGC ATAGATTCTG
  51  ATTCTTCTTT TAAGAGGACA CTGATAGACG TTCATGTTTT CAATTTCATC
 101  GCCAAGTTTC TGTTTAATAG AATTTTATTG AAGAAGAACC AAAACGATCC
 151  AAAATGGCTT CAAAACTTTT ACGACCAGGG AGATGGCAAA CATTTATGTG
 201  ATAAAGTTGA CTACAAGCGC TTGTGTTCGT TGCATTTTAC CCTTATTTAC
 251  TCTATTATTA ACATTCAACT CATCAAAATC AAGACAAACC AAACATTTGA
 301  ACCGCAGATA TTAAAATACG TATCTGTTCT GAAATTAATT GAACACATAC
 351  TTATCATCAT CGAAAGTCTG ATACATGTAC TTATTAGATT TGTATCGAAG
 401  CATAAACTAA TATGCATCAA CCGGAAAAAG GCGTACTGTC GAGTATACCT
 451  CGAAAGAGAA TTGAGTTTGA AGAAAACCTA CTTAAAGAAC TTTTACAGTG
 501  TAATAAGCGG TGTCCCAGAA AAAGAGTTAG GGGGTCTATT GAAAATACTC
 551  AAGATAGTTA TTCTATCATT GCTCGAGACA TTTGAAAGCA TTGAATGGCA
 601  GCACTTAAAA CCTTTCCTGG AAAAATTTCC GGCTCATGAA ATATCGCTTC
 651  AGAAGAAAAG GAAATATATA CAGGCGGCCT TATTAATTAC TGCCGAAAGA
 701  AATTTGATAG CGCGCTTTCG ATTGTCAAGA TGGTTCAATG AGACAGAAAA
 751  CATTTAATTT TTCTTTTGCA GTAGGAGGCG CATTATAAAA CACAAAAATA
 801  TCGAAAGCTC TTTCATTTCG GGGACAACAA CTTCAGTTGA AAATTACAGT
 851  GAACACAACA TCTTCCCCAA CAGACCTACA TTAAAACGCT TCTTCCGGAC
 901  TTGCCCATGA TTAACCTAAT CTTATACGAA CTGAATTAAA CTTTACGGTA
 951  TTACCGATAG GAAACTTCTA TTTTATGATT TTTTCGTTCG GGGACGGAAC
1001  GAACAGGAAA CAAAAAAAAA GGTACGATCC ATTGTATTCT CTACCCCCGT
1051  ATATAAAACT AAGCTGAACA AGCCTGTTGT TTTGCTTTAC TATTGCTACT
1101  ATTTTTGACG TAAACGCATT GACTAATTTC AGGTTTTTAT ATTCTTGACA
1151  CTAGCTAGAC CATAGTATCG AAGGATTCAA ATACACTAAA GTATCAGATA
1201  ATGTTCAGCC TACAAGACGT AATAACTACA ACCAAGACCA CCTTGGCAGC
1251  AATGCCAAAA GGTTACTTAC CAAAATGGTT ACTTTTCATT TCCATTGTAT
1301  CAGTCTTCAA TTCTATCCAG ACTTACGTTT CTGGTTTAGA ATTGACACGT
1351  AAAGTCTACG AAAGAAAACC CACTGAAACA ACCCATTTGA GTGCAAGAAC
1401  TTTCGGTACT TGGACCTTTA TTTCCTGTGT TATCAGATTC TATGGGGCTA
1451  TGTACTTGAA TGAACCACAC ATTTTCGAAT TGGTCTTCAT GTCTTATATG
1501  GTTGCCCTAT TCCACTTCGG CTCTGAATTA TTGATCTTTA GAACTTGTAA
1551  GTTGGGAAAG GGATTCATGG GTCCATTGGT TGTCTCAACC ACCTCTTTGG
1601  TTTGGATGTA CAAACAAAGA GAATACTACA CTGGTGTTGC TTGGTAA
```

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FIGURE 44. *YER044c* **Protein Sequence**

Nature 387:78-81 [97313264] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome V.

Dietrich, F. S., Mulligan, J., Hennessy, K., Yelton, M. A., Allen, E.,
Araujo, R., Aviles, E., Berno, A., Brennan, T., Carpenter, J., Chen,
E., Cherry, J. M., Chung, E., Duncan, M., Guzman, E., Hartzell, G., et al.

  YER044C Length: 148 March 26, 1999 16:40 Type: P Check: 3540 ..

     1 MFSLQDVITT TKTTLAAMPK GYLPKWLLFI SIVSVFNSIQ TYVSGLELTR

    51 KVYERKPTET THLSARTFGT WTFISCVIRF YGAMYLNEPH IFELVFMSYM

  101 VALFHFGSEL LIFRTCKLGK GFMGPLVVST TSLVWMYKQR EYYTGVAW

FIGURE 45.   Mouse EST with Similarity to *YER044c*

```
LOCUS       AI386195      455 bp    mRNA              EST        27-JAN-1999
DEFINITION  mq60h05.y1 Soares 2NbMT Mus musculus cDNA clone IMAGE:583161 5'
            similar to SW:YEN4_YEAST P40030 HYPOTHETICAL 17.1 KD PROTEIN IN
            SAH1-MEI4 INTERGENIC REGION. ;, mRNA sequence.
ACCESSION   AI386195
NID         g4199658
KEYWORDS    EST.
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
            Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 455)
  AUTHORS   Marra,M., Hillier,L., Kucaba,T., Martin,J., Beck,C., Wylie,T.,
            Underwood,K., Steptoe,M., Theising,B., Allen,M., Bowers,Y.,
            Person,B., Swaller,T., Gibbons,M., Pape,D., Harvey,N., Schurk,R.,
            Ritter,E., Kohn,S., Shin,T., Jackson,Y., Cardenas,M., McCann,R.,
            Waterston,R. and Wilson,R.
  TITLE     The WashU-NCI Mouse EST Project 1999
  JOURNAL   Unpublished (1999)
COMMENT
            Contact: Marra M/WashU-NCI Mouse EST Project 1999
            Washington University School of Medicine
            4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108, USA
            Tel: 314 286 1800
            Fax: 314 286 1810
            Email: mouseest@watson.wustl.edu
            This clone is available royalty-free through LLNL ; contact the
            IMAGE Consortium (info@image.llnl.gov) for further information.
            MGI:357809
            This read is a RESEQUENCE of a previously sequenced mouse clone
            This read has been verified (found to hit its original self in
the
            correct orientation)
            Seq primer: -40RP from Gibco
            High quality sequence stop: 455.
FEATURES            Location/Qualifiers
     source         1..455
                    /organism="Mus musculus"
                    /strain="C57BL/6J"
                    /note="Vector: pT7T3D-Pac (Pharmacia) with a modified
                    polylinker; Site_1: Not I; Site_2: Eco RI; 1st strand
cDNA
                    was primed with a Not I - oligo(dT) primer [5'
                    TGTTACCAATCTGAAGTGGGAGCGGCCGCGTTTTTTTTTTTTTTTTTTTTTTTTT
                    3']; double-stranded cDNA was ligated to Eco RI adaptors
                    (Pharmacia), digested with Not I and cloned into the Not
I
                    and Eco RI sites of the modified pT7T3 vector. RNA
                    provided by Dr. Bertrand Jordan. Library went through
two
                    rounds of normalization, and was constructed by Bento
                    Soares and M.Fatima Bonaldo."
                    /db_xref="taxon:10090"
                    /clone="IMAGE:583161"
                    /clone_lib="Soares 2NbMT"
                    /sex="male"
                    /tissue_type="Thymus"
                    /dev_stage="4 weeks"
                    /lab_host="DH10B"
```

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```
BASE COUNT       94 a    131 c    112 g    117 t      1 others
ORIGIN
        1 tgcggatgct gctgatactg ctgcagtagt actggatcgt caggcagagc gccctctctt
       61 ggaggggagt catgagccgc ttcctgaatg tgttacgaag ctggctggtt atggtgtcca
      121 ttatagccat ggggaacaca ctccagagct tccgagacca cacttttctc tacgagaagc
      181 tctacactgg caagccaaac cttgtgaatg gcctccaagc ccggaccttt gggatctgga
      241 cgctgctctc atcagtgatt cgctgcctct gtgccattga catccacaac aaaacactct
      301 atcacatcac actgtggaca ttcctcctcg ccctgngaca cttcctctca gagttgtttg
      361 tatttggaac agcagctccc acagttggtg tgctggcacc cctgatggta gcaagtttct
      421 caatcctggg catgctggtc gggctcccgt accta
//
```

FIGURE 45 (cont).

FIGURE 46.   **Human EST with Similarity to *YER044c***

```
LOCUS         W28235        839 bp    mRNA           EST         08-MAY-1996
DEFINITION    43h8 Human retina cDNA randomly primed sublibrary Homo sapiens
              cDNA, mRNA sequence.
ACCESSION     W28235
NID           g1308183
KEYWORDS      EST.
SOURCE        human.
  ORGANISM    Homo sapiens
              Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
              Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE     1  (bases 1 to 839)
  AUTHORS     Macke, J., Smallwood, P. and Nathans, J.
  TITLE       Adult Human Retina cDNA
  JOURNAL     Unpublished (1996)
COMMENT

              Contact: Dr. Jeremy Nathans
              Dr. Jeremy Nathans, Dept. of Molecular Biology and Genetics
              Johns Hopkins School of Medicine
              725 North Wolfe Street, Baltimore, MD 21205
              Tel: 410 955 4678
              Fax: 410 614 0827
              Email: jeremy_nathans@qmail.bs.jhu.edu
              Clones from this library are NOT available.
              PCR PRimers
              FORWARD: CTTTTGAGCAAGTTCAGCCTGGTTAAGT
              BACKWARD: GAGGTGGCTTATGAGTATTTCTTCCAGGGTAA
              Seq primer: GGGTAAAAAGCAAAAGAATT.
FEATURES              Location/Qualifiers
     source           1..839
                      /organism="Homo sapiens"
                      /note="Organ: eye; Vector: lambda gt10; Site_1: EcoRI;
                      Site_2: EcoRI; The library used for sequencing was a
                      sublibrary derived from a human retina cDNA library.
                      Inserts from retina cDNA library DNA were isolated,
                      randomly primed, PCR amplified, size-selected, and
cloned
                      into lambda gt10.  Individual plaques were arrayed and
                      used as templates for PCR amplification, and these PCR
                      products were used for sequencing."
                      /db_xref="taxon:9606"
                      /clone_lib="Human retina cDNA randomly primed
sublibrary"
                      /sex="mixed (males and females)"
                      /tissue_type="retina"
                      /dev_stage="adult"
                      /lab_host="E. coli strain K802"
BASE COUNT      127 a    141 c    136 g    140 t    295 others
ORIGIN
        1 gnnnnnngnn nnnnnnnnnt tnttgagnac cgcagtngca gcagcagcag ccgctgncgc
       61 aaacaagccc tcccacgttt gaggggagtc atgagccgtt tcctgaatgt gttaagaagt
      121 tggctggtta tggtgtccat catagccatg gggaacacgc tgcagagctt ccgagaccac
      181 acttttctct atgaaaagct ctacactggc aagccaaacc ttgtgaatgg cctccaagct
      241 cggacctttg ggatctggac gctgctctca tcagtgattc gctgcctctg tgccattgac
      301 attcacaaca agacgctcta tcacatcaca ctctggacct tcctccttgc cctggggcat
      361 ttcctctctg agttgtttgt cttatggaac tgcagctccc acgattggng tcctggcanc
      421 cctgatggtg gnaagtttct ccatcctggg tattgtggtc ggctccngta ttttagaagt
      481 agaaccagtt ccagacagaa gaagagaact gaggcagaat atcaacccca gggtggatca
      541 antgggttac aagtggttna aaannnnnnn nnnnnnnnnc nnnntnntnt naannnnnnn
      601 nnnnnnnnnn nnnnnnnna nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
```

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```
661 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
721 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn
781 nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnnnn nnnnnnnc
//
```

FIGURE 46 (cont).

FIGURE 47.   Rat EST with Similarity to YER044c

```
LOCUS       AI172515      475 bp    mRNA            EST        11-FEB-1999
DEFINITION  UI-R-C2p-nu-d-02-0-UI.s1 UI-R-C2p Rattus norvegicus cDNA clone
            UI-R-C2p-nu-d-02-0-UI 3', mRNA sequence.
ACCESSION   AI172515
NID         g3712555
KEYWORDS    EST.
SOURCE      Norway rat.
  ORGANISM  Rattus norvegicus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
            Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Rattus.
REFERENCE   1  (bases 1 to 475)
  AUTHORS   Bonaldo,M.F., Lennon,G. and Soares,M.B.
  TITLE     Normalization and subtraction: two approaches to facilitate gene
            discovery
  JOURNAL   Genome Res. 6 (9), 791-806 (1996)
  MEDLINE   97044477
COMMENT

            Contact: Soares, MB
            Program for Rat Gene Discovery and Mapping
            University of Iowa
            451 Eckstein Medical Research Building Iowa City, IA 52242, USA
            Tel: 319 335 8250
            Fax: 319 335 9565
            Email: msoares@blue.weeg.uiowa.edu
            The sequence tag present in the cDNA between the NotI site and
the
            oligo-dT track served to identify it as a clone from the
normalized
            adult Placenta library. cDNA Library Preparation: M. Fatima
            Bonaldo, Ph.D. Clone distribution: clones will be available
through
            Research Genetics
            Seq primer: M13 Forward.
FEATURES             Location/Qualifiers
     source          1..475
                     /organism="Rattus norvegicus"
                     /strain="Sprague-Dawley"
                     /note="Vector: pT7T3D-Pac (Pharmacia) with a modified
                     polylinker; Site_1: Not I; Site_2: Eco RI; The UI-R-C2p
                     library is a subtracted library derived from the UI-R-C1
                     library, which is a subtracted library derived from the
                     UI-R-C0 library.  The UI-R-C0 library consisted of a
                     mixture of individually tagged normalized  libraries
                     constructed from rat placenta, adult lung, brain, liver,
                     kidney,  heart, spleen, ovary, muscle, 8, 12 and 18-day
                     embryo. The tag is a string of  3-5 nucleotides present
                     between the Not I site and the oligo-dT track which
allows
                     identification of the library of origin of a clone
within
                     the mixture. The subtracted library (UI-R-C2p) was
                     constructed as follows: PCR amplified cDNA inserts from
                     UI-R-C1 clones from which 3' ESTs had been derived was
                     used as a driver in a hybridization with the UI-R-C1
                     library in the form of single-stranded circles. The
                     remaining single-stranded circles (subtracted library)
was
                     purified by hydroxyapatite column chromatography,
                     converted to double-stranded circles and electroporated
```

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into DH10B bacteria (Life Technologies) to generate the
UI-R-C2p library. This procedure has been previously
described (Bonaldo, Lennon and Soares, Genome Research

6:

791-806, 1996)"
/db_xref="taxon:10116"
/clone="UI-R-C2p-nu-d-02-0-UI"
/clone_lib="UI-R-C2p"
/dev_stage="adult"
/lab_host="DH10B (Life Technologies)"

BASE COUNT   115 a   112 c   126 g   119 t   3 others
ORIGIN
        1 tttttttttt tttttttctg tctggatact ggttctgctt ctaggtaccg gagcccaact
       61 agcatacccà ggattgagaa acttgctacc atcaagggtg ccagcacacc aactgtggga
      121 gccgctgttc caaatacaaa caactccgag aggaagtgtc ccagggcaag gaggaatgtc
      181 cacagtgtga tgtgatagag tgttttgttg tggatgtcaa tggcacagag gcagcgaatc
      241 actgaagaga gcagcgtcca gatcccaaag gtccgggctt ggaggccatt cacaaggttt
      301 ggtttgccag tgtanagctt ttcatanaga aaagtgtggt ctcggaagct ctggagcgtg
      361 ttncccatgg ctatgatgga caccataacc agccagcttc gtagcacatt caggaagcgg
      421 ctcatgactc ccctcaaaga gagggcgctc tgcctgaccc tcgtgccgaa ttctt
//

FIGURE 47 (cont)

FIGURE 48. YLR100w DNA Sequence

Sequence contains 800bp of 5' promoter sequence.
Symbols: 1 to: 1844 from: chr12.gcg   ck: 2436, 341011 to: 342854
Chromosome XII Sequence
Nature 387:87-90 [97313267] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XII.
Johnston, M., Hillier, L., Riles, L., Albermann, K., Andre, B.,
Ansorge, W., Benes, V., Bruckner, M., Delius, H., Dubois, E., . . .

gcgseq.tmp.10136 Length: 1844 March 26, 1999 15:19 Type: N Check: 2071
..

```
   1  ACGTACAAAA AAGAGCACGC TGCTTTATTT ATACTTTTGT GCCACAAGAA
  51  TGATCAACAT CAACATAAAT ATCAACTAGT ATCTGCAACA CATCTGCTCC
 101  ACGGAACTAA ACCCGTTGAG CAGTGCCCCG TGGAAACGTA AACTATCGCA
 151  AATTGGGATT AACAAGCCAA AAACAGCCAA GCAAGATTCA CGAAACCGCG
 201  CCTCGTTTGG ACCCCGAAGG CCCATTTAAC GGCCGGCCGT TACAAGCAAG
 251  ATCGGCAGAG CAAACCACTC CCCAGCACCA CAGCACATCA CTGCACGAGC
 301  AACAATAACT AGAACATGGC AGATAGCGAG GATACCTCTG TGATCCTGCA
 351  GGGCATCGAC ACAATCAACA GCGTGGAGGG CCTGGAAGAA GATGGTTACC
 401  TCAGCGACGA GGACACGTCA CTCAGCAACG AGCTCGCAGA TGCACAGCGT
 451  CAATGGGAAG AGTCGCTGCA ACAGTTGAAC AAGCTGCTCA ACTGGGTCCT
 501  GCTGCCCCTG CTGGGCAAGT ATATAGGTAG GAGAATGGCC AAGACTCTAT
 551  GGAGTAGGTT CATTGAACAC TTTGTATAAG TGTTTGTTGT TTATGTATCC
 601  GCATATAGCA GTTATAACAG ATAAATGGCA CTTTTCGCAC ACCCGTTGTT
 651  TTATCTCCGA TAGTACGTGG GCCTTTATTT ATGGTCGTTT AACGAAAGAA
 701  CGGCATCTTG AATTGAGCAG GTATTTAAAA GATAGGACGA GAAACAAGCA
 751  CATGATCTGT GTCGAAAAAA AGTAGCAAAG AGAAAAAGTA GGAGGATAGG
 801  ATGAACAGGA AAGTAGCTAT CGTAACGGGT ACTAATAGTA ATCTTGGTCT
 851  GAACATTGTG TTCCGTCTGA TTGAAACTGA GGACACCAAT GTCAGATTGA
 901  CCATTGTGGT GACTTCTAGA ACGCTTCCTC GAGTGCAGGA GGTGATTAAC
 951  CAGATTAAAG ATTTTTACAA CAAATCAGGC CGTGTAGAGG ATTTGGAAAT
1001  AGACTTTGAT TATCTGTTGG TGGACTTCAC CAACATGGTG AGTGTCTTGA
1051  ACGCATATTA CGACATCAAC AAAAAGTACA GGGCGATAAA CTACCTTTTC
1101  GTGAATGCTG CGCAAGGTAT CTTTGACGGT ATAGATTGGA TCGGAGCGGT
1151  CAAGGAGGTT TTCACCAATC CATTGGAGGC AGTGACAAAT CCGACATACA
1201  AGATACAACT GGTGGGCGTC AAGTCTAAAG ATGACATGGG GCTTATTTTC
1251  CAGGCCAATG TGTTTGGTCC GTACTACTTT ATCAGTAAAA TTCTGCCTCA
1301  ATTGACCAGG GGAAAGGCTT ATATTGTTTG GATTTCGAGT ATTATGTCCG
1351  ATCCTAAGTA TCTTTCGTTG AACGATATTG AACTACTAAA GACAAATGCC
1401  TCTTATGAGG GCTCCAAGCG TTTAGTTGAT TTACTGCATT TGGCCACCTA
1451  CAAAGACTTG AAAAAGCTGG GCATAAATCA GTATGTAGTT CAACCGGGCA
1501  TATTTACAAG CCATTCCTTC TCCGAATATT TGAATTTTTT CACCTATTTC
1551  GGCATGCTAT GCTTGTTCTA TTTGGCCAGG CTGTTGGGGT CTCCATGGCA
1601  CAATATTGAT GGTTATAAAG CTGCCAATGC CCCAGTATAC GTAACTAGAT
1651  TGGCCAATCC AAACTTTGAG AAACAAGACG TAAAATACGG TTCTGCTACC
1701  TCTAGGGATG GTATGCCATA TATCAAGACG CAGGAAATAG ACCCTACTGG
1751  AATGTCTGAT GTCTTCGCTT ATATACAGAA GAAGAAACTG GAATGGGACG
1801  AGAAACTGAA AGATCAAATT GTTGAAACTA GAACCCCCAT TTAA
```

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FIGURE 49. *YLR100w* **Protein Sequence**

Nature 387:87-90 [97313267] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XII.

Johnston, M., Hillier, L., Riles, L., Albermann, K., Andre, B.,
Ansorge, W., Benes, V., Bruckner, M., Delius, H., Dubois, E.,
Dusterhoft, A., Entian, K. D., Floeth, M., Goffeau, A., Hebling, U., et al.

YLR100W Length: 347 March 26, 1999 15:20 Type: P Check: 2853 ..

```
    1   MNRKVAIVTG TNSNLGLNIV FRLIETEDTN VRLTIVVTSR TLPRVQEVIN

   51   QIKDFYNKSG RVEDLEIDFD YLLVDFTNMV SVLNAYYDIN KKYRAINYLF

  101   VNAAQGIFDG IDWIGAVKEV FTNPLEAVTN PTYKIQLVGV KSKDDMGLIF

  151   QANVFGPYYF ISKILPQLTR GKAYIVWISS IMSDPKYLSL NDIELLKTNA

  201   SYEGSKRLVD LLHLATYKDL KKLGINQYVV QPGIFTSHSF SEYLNFFTYF

  251   GMLCLFYLAR LLGSPWHNID GYKAANAPVY VTRLANPNFE KQDVKYGSAT

  301   SRDGMPYIKT QEIDPTGMSD VFAYIQKKKL EWDEKLKDQI VETRTPI
```

FIGURE 50. **Human EST with Similarity to *YLR100w***

```
LOCUS        R92053       454 bp    mRNA            EST        25-AUG-1995
DEFINITION   yp96c01.r1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA
clone
             IMAGE:195264 5', mRNA sequence.
ACCESSION    R92053
NID          g959593
KEYWORDS     EST.
SOURCE       human.
  ORGANISM   Homo sapiens
             Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
             Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE    1  (bases 1 to 454)
  AUTHORS    Hillier,L., Clark,N., Dubuque,T., Elliston,K., Hawkins,M.,
             Holman,M., Hultman,M., Kucaba,T., Le,M., Lennon,G., Marra,M.,
             Parsons,J., Rifkin,L., Rohlfing,T., Soares,M., Tan,F.,
             Trevaskis,E., Waterston,R., Williamson,A., Wohldmann,P. and
             Wilson,R.
  TITLE      The WashU-Merck EST Project
  JOURNAL    Unpublished (1995)
COMMENT
             Contact: Wilson RK
             Washington University School of Medicine
             4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108
             Tel: 314 286 1800
             Fax: 314 286 1810
             Email: est@watson.wustl.edu
             Insert Size: 1067
             High quality sequence stops: 337
             Source: IMAGE Consortium, LLNL
             This clone is available royalty-free through LLNL ; contact the
             IMAGE Consortium (info@image.llnl.gov) for further information.
             Insert Length: 1067    Std Error: 0.00
             Seq primer: M13RP1
             High quality sequence stop: 337.
FEATURES             Location/Qualifiers
     source          1..454
                     /organism="Homo sapiens"
                     /note="Organ: Liver and Spleen; Vector: pT7T3D
(Pharmacia)
                     with a modified polylinker; Site_1: Pac I; Site_2: Eco
RI;
                     1st strand cDNA was primed with a Pac I - oligo(dT)
primer
                     [5' AACTGGAAGAATTAATTAAAGATCTTTTTTTTTTTTTTTTTTT 3'],
                     double-stranded cDNA was ligated to Eco RI adaptors
                     (Pharmacia), digested with Pac I and cloned into the Pac
I
                     and Eco RI sites of the modified pT7T3 vector.  Library
                     went through one round of normalization. Library
                     constructed by Bento Soares and M.Fatima Bonaldo."
                     /db_xref="GDB:3764314"
                     /db_xref="taxon:9606"
                     /clone="IMAGE:195264"
                     /clone_lib="Soares fetal liver spleen 1NFLS"
                     /sex="male"
                     /dev_stage="20 week-post conception fetus"
                     /lab_host="DH10B (ampicillin resistant)"
BASE COUNT      115 a    111 c     96 g     129 t      3 others
```

```
ORIGIN
        1 tttgagacca atgtctttgg ccattttatc ctgattcggg aactggagcc tctcctctgt
       61 cacagtgaca atccatctca gctcatctgg acatcatctc gcagtgcaag gaaatctaat
      121 ttcagcctcg aggacttcca gcacagcaaa ggcaaggaac cctacagctc ttccaaatat
      181 gccactgacc ttttgagtgt ggctttgaac aggaacttca accagcaggg tctctattcc
      241 aatgtggcct gtccaggtac agcattgacc aatttgacat atggaattct gcctccgttt
      301 atatggacgc tgttggatgc cggcaatatt gctacttcgc ttttttggca aatggcattc
      361 actttggaca ccatataatg ggaacaggaa gntatgggta tgggnttttc ccaccaaaag
      421 gctggaatcn tttcaatcct ctggatccaa atat
//
```

FIGURE 50 (cont).

FIGURE 51. **Mouse EST with Similarity to _YLR100w_**

```
LOCUS       AI226514      1039 bp     mRNA              EST        29-OCT-1998
DEFINITION  uj07d08.y1 Sugano mouse liver mlia Mus musculus cDNA clone
            IMAGE:1891215 5' similar to TR:Q62904 Q62904 OVARIAN-SPECIFIC
            PROTEIN. ;, mRNA sequence.
ACCESSION   AI226514
NID         g3809567
KEYWORDS    EST.
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
            Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (bases 1 to 1039)
  AUTHORS   Marra,M., Hillier,L., Allen,M., Bowles,M., Dietrich,N.,
Dubuque,T.,
            Geisel,S., Kucaba,T., Lacy,M., Le,M., Martin,J., Morris,M.,
            Schellenberg,K., Steptoe,M., Tan,F., Underwood,K., Moore,B.,
            Theising,B., Wylie,T., Lennon,G., Soares,B., Wilson,R. and
            Waterston,R.
  TITLE     The WashU-HHMI Mouse EST Project
  JOURNAL   Unpublished (1996)
COMMENT
            Contact: Marra M/Mouse EST Project
            WashU-HHMI Mouse EST Project
            Washington University School of MedicineP
            4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108
            Tel: 314 286 1800
            Fax: 314 286 1810
            Email: mouseest@watson.wustl.edu
            This clone is available royalty-free through LLNL ; contact the
            IMAGE Consortium (info@image.llnl.gov) for further information.
            MGI:975539
            Seq primer: custom primer used
            High quality sequence stop: 509.
FEATURES          Location/Qualifiers
     source       1..1039
                  /organism="Mus musculus"
                  /strain="C57BL"
                  /note="Organ: liver; Vector: pME18S-FL3; Site_1: DraIII
                  (CACTGTGTG); Site_2: DraIII (CACCATGTG); 1st strand cDNA
                  was primed with an oligo(dT) primer
                  [ATGTGGCCTTTTTTTTTTTTTTTTTTT]; double-stranded cDNA was
                  ligated to a  DraIII adaptor [TGTTGGCCTACTGG], digested
                  and cloned into distinct DraIII sites of the pME18S-FL3
                  vector (5' site CACTGTGTG, 3' site CACCATGTG). XhoI
should
                  be used to isolate the cDNA insert.  Size selection was
                  performed to exclude fragments <1.5kb.  Library
                  constructed by Dr. Sumio Sugano (University of Tokyo
                  Institute of Medical Science).  Custom primers  for
                  sequencing: 5' end primer CTTCTGCTCTAAAAGCTGCG and 3'
end
                  primer CGACCTGCAGCTCGAGCACA."
                  /db_xref="taxon:10090"
                  /clone="IMAGE:1891215"
                  /clone_lib="Sugano mouse liver mlia"
                  /sex="female"
                  /dev_stage="adult"
                  /lab_host="DH10B"
```

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```
BASE COUNT       245 a     267 c     251 g     272 t       4 others
ORIGIN
        1 ggctaagaga accccggtgc agttctactt cggtgcaggg cgtggaagat gcggaaggtg
       61 gttttgatca ccggggcgag cagtggcatt gggctagccc tttgcggtcg actgctggca
      121 gaagacgatg acctccacct gtgtttggcg tgtaggaacc tgagcaaagc aagagctgtt
      181 cgagataccc tgctggcctc tcacccctcc gccgaagtca gcatcgtgca gatggatgtc
      241 agcagcctgc agtcggtggt ccggggtgca gaggaagtca agcaaaagtt tcaaagatta
      301 gactacttat atctgaatgc tggaatcctg cctaatccac aattcaacct caaggcattt
      361 ttctgcggca tcttttcaag aaatgtgatt catatgttca ccacagcgga aggaattttg
      421 acccagaatg actcggtcac tgccgacggg ttgcaggagg tgtttgaaac caatctcttt
      481 ggccacttta ttctgattcg ggaactggaa ccacttctct gccatgccga caacccctct
      541 cagctcatct ggacgtcctc tcgcaatgca aagaaggcta acttcagcct ggaggacata
      601 cagcacttca aaggcccgga accctacagc tctttccaat atgctaccga cctcctgaat
      661 gtggctntga acagggaatt caaaccagaa ggtctggtat tcagtggtga ttgccgaggg
      721 cgtctgatga ccaatatgac gtatggaaat ttgccttcct ttatcctgac cgtggttcta
      781 cccttaagtg ggctccttcg ctttttttgaa aatgccctca cctgggaccc cgtaccactg
      841 atcaaaagct ctgggtgtgt ttctttcaca tataaccgga ggctttttatt ctttgaccaa
      901 atacgcgagc tccaccttgg tagtgggact atataccgac cggtcccacg aatgcactca
      961 tttaacacct tgtcaaaact ttttatagtt ttacctgttg tgataacgtg gtgntacccc
     1021 cttcgtantt gnaataccc
//
```

FIGURE 51 (cont).

FIGURE 52.   Mouse EST with Similarity to *YLR100w*


```
LOCUS        AI528381       837 bp      mRNA              EST         18-MAR-1999
DEFINITION   ui96g06.y1 Sugano mouse liver mlia Mus musculus cDNA clone
             IMAGE:1890298 5' similar to TR:Q62904 Q62904 OVARIAN-SPECIFIC
             PROTEIN. ;, mRNA sequence.
ACCESSION    AI528381
NID          g4442516
KEYWORDS     EST.
SOURCE       house mouse.
  ORGANISM   Mus musculus
             Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Mammalia;
             Eutheria; Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE    1  (bases 1 to 837)
  AUTHORS    Marra,M., Hillier,L., Kucaba,T., Martin,J., Beck,C., Wylie,T.,
             Underwood,K., Steptoe,M., Theising,B., Allen,M., Bowers,Y.,
             Person,B., Swaller,T., Gibbons,M., Pape,D., Harvey,N., Schurk,R.,
             Ritter,E., Kohn,S., Shin,T., Jackson,Y., Cardenas,M., McCann,R.,
             Waterston,R. and Wilson,R.
  TITLE      The WashU-NCI Mouse EST Project 1999
  JOURNAL    Unpublished (1999)
COMMENT

             Contact: Marra M/WashU-NCI Mouse EST Project 1999
             Washington University School of Medicine
             4444 Forest Park Parkway, Box 8501, St. Louis, MO 63108, USA
             Tel: 314 286 1800
             Fax: 314 286 1810
             Email: mouseest@watson.wustl.edu
             This clone is available royalty-free through LLNL ; contact the
             IMAGE Consortium (info@image.llnl.gov) for further information.
             MGI:974622
             Possible reversed clone: similarity on wrong strand
             Seq primer: custom primer used
             High quality sequence stop: 429.
FEATURES             Location/Qualifiers
     source          1..837
                     /organism="Mus musculus"
                     /strain="C57BL"
                     /note="Organ: liver; Vector: pME18S-FL3; Site_1: DraIII
                     (CACTGTGTG); Site_2: DraIII (CACCATGTG); 1st strand cDNA
                     was primed with an oligo(dT) primer
                     [ATGTGGCCTTTTTTTTTTTTTTTTTT]; double-stranded cDNA was
                     ligated to a  DraIII adaptor [TGTTGGCCTACTGG], digested
                     and cloned into distinct DraIII sites of the pME18S-FL3
                     vector (5' site CACTGTGTG, 3' site CACCATGTG). XhoI
should
                     be used to isolate the cDNA insert.  Size selection was
                     performed to exclude fragments <1.5kb.  Library
                     constructed by Dr. Sumio Sugano (University of Tokyo
                     Institute of Medical Science).  Custom primers  for
                     sequencing: 5' end primer CTTCTGCTCTAAAAGCTGCG and 3'
end
                     primer CGACCTGCAGCTCGAGCACA."
                     /db_xref="taxon:10090"
                     /clone="IMAGE:1890298"
                     /clone_lib="Sugano mouse liver mlia"
                     /sex="female"
                     /dev_stage="adult"
                     /lab_host="DH10B"
```

```
BASE COUNT        191 a     222 c     212 g     208 t        4 others
ORIGIN
        1 ggctaagaga accccggtgc agttctactt cggtgcaggg cgtggaagat gcggaaggtg
       61 gttttgatca ccggggcgag cagtggcatt gggctagccc tttgcggtcg actgctggca
      121 gaagacgatg acctccacct gtgtttggcg tgtaggaacc tgagcaaagc aagagctgtt
      181 cgagataccc tgctggcctc tcacccctcc gccgaagtca gcatcgtgca gatggatgtc
      241 agcagcctgc agtcggtggt ccggggtgca gaggaagtca agcaaaagtt tcaaagatta
      301 gactacttat atctgaatgc tggaatcctg cctaatccac aattcaacct caaggcattt
      361 ttctgcggca tcttttcaag aaatgtgatt catatgttca ccacagcgga aggaattttg
      421 acccagaatg actcggtcac tgccgaccgg ttgcaggagg tgtttgaaac caatctctct
      481 tgccacttta ttctgattcg ggaactggaa ccacttctct tgcatgcgga caacccctct
      541 cagctcatct ggacgtcctc tcgcaatgca nagaaggcta acttcagcct ggaggacatn
      601 cagcactcca tagggcccgg accctacagc tctttccaat atgctaccga cctcctgaat
      661 gtggctttga acangaatnt caaccagaag ggtctgtatt ccagtcgcat gtgcccaggc
      721 gtcgtgatga ccaatatgac gtatggaatc ttgcctccct tttatctgga cgtgctccta
      781 cccatgatgg tgctccttcg cttctttggt aatgcgctta ctgggacacc gtacaac
//
```

FIGURE 52 (cont).

FIGURE 53.   Mouse Gene with Similarity to *YLR100w*

```
LOCUS       3319971       334 aa                      14-JUL-1998
DEFINITION  17-beta-hydroxysteroid dehydrogenase type 7.
ACCESSION   3319971
PID         g3319971
DBSOURCE    EMBL: locus MMY15733, accession Y15733
KEYWORDS    .
SOURCE      house mouse.
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Rodentia; Sciurognathi; Muridae; Murinae; Mus.
REFERENCE   1  (residues 1 to 334)
  AUTHORS   Nokelainen,P., Peltoketo,H., Vihko,R. and Vihko,P.
  TITLE     Expression cloning of a novel estrogenic mouse 17
            beta-hydroxysteroid dehydrogenase/17-ketosteroid reductase
            (m17HSD7), previously described as a prolactin receptor-
            associated protein (PRAP) in rat
  JOURNAL   Mol. Endocrinol. 12 (7), 1048-1059 (1998)
  MEDLINE   98322544
REFERENCE   2  (residues 1 to 334)
  AUTHORS   Nokelainen,P.A.
  TITLE     Direct Submission
  JOURNAL   Submitted (27-NOV-1997) P.A. Nokelainen, University of Oulu,
            Biocenter Oulu, WHO Collaborating Centre for Research on
            Reproductive Health Department of Clinical Chemistry, Kajaanintie
            50, FIN-90220 Oulu, FINLAND
FEATURES             Location/Qualifiers
     source          1..334
                     /organism="Mus musculus"
                     /strain="BALB/c"
                     /db_xref="taxon:10090"
                     /tissue_type="mammary gland"
                     /cell_type="epithelial cell derived from mammary gland
of
                     a pregnant mouse"
                     /clone_lib="cDNA library prepared from poly(A)-enriched
                     RNA isolated from HC11 cell line"
                     /clone="m17HSD7.1"
                     /clone="m17HSD7.2"
     Protein         1..334
                     /product="17-beta-hydroxysteroid dehydrogenase type 7"
     CDS             1..334
                     /gene="HSD17B7"
                     /db_xref="SPTREMBL:O88736"
                     /coded_by="Y15733:64..1068"
ORIGIN
        1 mrkvvlitga ssgiglalcg rllaedddlh lclacrnlsk aravrdtlla shpsaevsiv
       61 qmdvsslqsv vrgaeevkqk fqrldylyln agilpnpqfn lkaffcgifs rnvihmftta
      121 egiltqndsv tadglqevfe tnlfghfili relepllcha dnpsqliwts srnakkanfs
      181 lediqhskgp epyssskyat dllnvalnrn fnqkglyssv mcpgvvmtnm tygilppfiw
      241 tlllpimwll rffvnaltvt pyngaealvw lfhqkpesln pltkyasats gfgtnyvtgq
      301 kmdidedtae kfyevllele krvrttvqks dhps
//
```

FIGURE 54. *YER034w* DNA Sequence

Sequence contains 559bp of 5' promoter sequence.
Symbols: 1 to: 1117 from: chr5.gcg   ck: 9036, 221286 to: 222402
Chromosome V Sequence
Nature 387:78-81 [97313264] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome V.
Dietrich, F. S., Mulligan, J., Hennessy, K., Yelton, M. A., Allen, E.,
Araujo, R., Aviles, E., Berno, A., Brennan, T., Carpenter, J., Chen, . . .

gcgseq.tmp.6597 Length: 1117 March 26, 1999 16:54 Type: N Check: 5026 ..

```
   1  TGATGAAATA TTCCAGTTAT GCGTGTGCGT CTTGTGATGC AGATCCTTTT
  51  GGGCAAAAAC AGTTGGTTTG TGCGAAAACG CAAGGTAATA AATAGGCTTA
 101  AAGGAACTAA AAAAAAAAAA AGGAAAATAA CCAGCTAAGA TTTAAGGTAC
 151  AAGAAAGCGG TTGCACCTCA AGTAATGATA GTTATTAAAC CTTGGATTGG
 201  ACCAGATGTT TAAAATTGTT TTCAATAGTA GATTTGCAGT CGTAAATGCG
 251  TTCTCAGCAA TATCATATTG TGTTTATGAA GTATTACCAA ACGGGTAGAA
 301  GAACGGTTTA AGAGAATATG TCCGGATAAA GCGATCAGGA GAAAAGCTTA
 351  AAACCCAAAG TGGTCAATCT GCAGCCCATT TAGGCACTCT GCATTTAACC
 401  GATACCCGGA TTGAAGAAAG CTGGCGGGTG TATGGGTGAA GGAGAAGAAA
 451  GGAAGTGATT AGGAGAAACC TCATGGAGAT GAGCACATGC TACAACTAAT
 501  AACGTTATTC TACTTAAAAC GAGCAAAACA AAAAAAAAAA CAAGACAATT
 551  GAAAACGCAA TGGATGCATT CAGCTTAAAG AAGGATAATC GAAAAAAATT
 601  TCAAGATAAA CAGAAATTGA AAAGAAAACA TGCCACACCC AGTGATAGAA
 651  AGTACCGGCT ATTGAACCGC CAAAAAGAAG AGAAAGCTAC CACAGAGGAG
 701  AAAGATCAAG ACCAAGAACA GCCCGCCCTG AAGTCAAACG AGGACAGGTA
 751  CTATGAGGAC CCGGTACTCG AGGACCCGCA TTCTGCAGTC GCCAATGCAG
 801  AGTTGAACAA GGTGCTAAAA GACGTCCTCA AAAATCGGCT CCAGCAGAAC
 851  GACGACGCCA CAGCCGTCAA TAATGTTGCT AATAAAGATA CTTTGAAAAT
 901  CAAAGACCTC AAGCAGATGA ATACGGATGA GCTCAATCGT TGGCTCGGAC
 951  GGCAGAATAC AACATCGGCT ATAACAGCGG CTGAGCCCGA ATCATTAGTC
1001  GTTCCCATTC ACGTACAAGG TGATCATGAT CGTGCGGGCA AGAAGATCAG
1051  TGCCCCTTCG ACCGATCTAC CGGAAGAACT AGAGACCGAT CAGGATTTCC
1101  TTGATGGACT GCTCTAA
```

FIGURE 55. *YER034w* Protein Sequence

Nature 387:78-81 [97313264] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome V.

Dietrich, F. S., Mulligan, J., Hennessy, K., Yelton, M. A., Allen, E.,
Araujo, R., Aviles, E., Berno, A., Brennan, T., Carpenter, J., Chen,
E., Cherry, J. M., Chung, E., Duncan, M., Guzman, E., Hartzell, G., et al.

```
YER034W  Length: 185  March 26, 1999 16:55  Type: P  Check: 3501  ..

    1  MDAFSLKKDN RKKFQDKQKL KRKHATPSDR KYRLLNRQKE EKATTEEKDQ

   51  DQEQPALKSN EDRYYEDPVL EDPHSAVANA ELNKVLKDVL KNRLQQNDDA

  101  TAVNNVANKD TLKIKDLKQM NTDELNRWLG RQNTTSAITA AEPESLVVPI

  151  HVQGDHDRAG KKISAPSTDL PEELETDQDF LDGLL
```

FIGURE 56. *YKL077w* DNA Sequence

Sequence contains 1200bp of 5' promoter sequence.
Symbols: 1 to: 2379   from: chr11.gcg   ck: 9298, 289895 to: 292273
Chromosome XI Sequence
Nature 387:98-102 [97313270] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XV.
Dujon, B., Albermann, K., Aldea, M., Alexandraki, D., Ansorge, W.,
Arino, J., Benes, V., Bohn, C., Bolotin-Fukuhara, M., Bordonne, R., . . .

gcgseq.tmp.4920 Length: 2379 March 26, 1999 16:48 Type: N Check: 4118 ..

```
   1  GAAAGGAAGC TATAGTAATG GGGCTTCAGG AACTTTATGA ATTGGGTGCT
  51  CTTGACACTC GTGGAAAGAT AACTAAACGG GGTCAACAAA TGGCTCTGTT
 101  ACCGCTACAA CCGCATTTAA GTAGTGTCTT AATTAAAGCC AGTGAAGTCG
 151  GATGTTTGAG TCAGGTCATT GATATCGTCT CTTGCCTTAG TGTGGAAAAT
 201  TTACTGTTGA ATCCGTCACC AGAAGAAAGA GATGAGGTGA ACGAGCGTCG
 251  TTTGTCCTTA TGCAACGCTG GTAAAAGGTA TGGTGACCTT ATCATGCTGA
 301  AAGAGCTTTT TGATATCTAT TTCTACGAAC TAGGGAAAAG TCAAGATGCA
 351  AGCTCTGAAA GAAATGATTG GTGTAAAGGA TTGTGTATTT CGATACGTGG
 401  GTTTAAAAAT GTAATTCGTG TCAGAGACCA GTTAAGAGTT TATTGTAAGC
 451  GTTTGTTTTC TTCAATCAGT GAAGAGGATG AAGAATCCAA AAAGATTGGT
 501  GAAGATGGCG AGCTAATTTC GAAAATTTTA AAGTGTTTCT TAACTGGGTT
 551  TATCAAGAAT ACAGCTATAG GGATGCCAGA CAGGTCTTAT AGAACTGTTT
 601  CCACTGGAGA GCCGATAAGC ATTCATCCAT CATCTATGCT ATTTATGAAT
 651  AAAAGCTGCC CCGGTATAAT GTACACGGAG TATGTCTTTA CTACGAAGGG
 701  ATATGCCAGA AATGTTAGTA GGATTGAACT TTCATGGTTA CAAGAAGTTG
 751  TCACTAATGC AGCCGCTGTA GCAAAGCAAA AAGTTTCTGA TTCAAAATAA
 801  GTCACCTACT CTTAGCGCAT TTTTATTGTA TATAAAGGCA TTTAATGTAA
 851  TTTATAGAGC ATTATAAATC GTAACAACTA CTGCAGTATG AGTTTCATGG
 901  ATTCATTTCT CAATATCTTA TGAATATACA CAGGTATATA TGTATATTCA
 951  TGTTAAACGC CTTTCGAATT GTTCGTTGGC TTTTTTTGTG AAATTATCTC
1001  GGGAAAAGGG CGAAATTATA TTATTTTGCC GTTGACATTT TGAAAAGGAA
1051  TAAAAGATCA TGAAAAAAAT AAGAAAGGCA ATTCGACGCA TTTCTCTCAG
1101  CAAGCTATTC TTTACTTTTG AAGAACAAAA TATTTTAGCA AAAAGGTTAA
1151  GACAATATAG TCGGAAGCAG TTCTGCGGGA TCTGAAGGAA TTGCGGAATA
1201  ATGAGATTTC ACGATAGTAT ACTTATCTTC TTTTCTTTGG CATCGCTTTA
1251  TCAACATGTT CATGGTGCAA GACAAGTCGT TCGTCCAAAG GAGAAAATGA
1301  CTACTTCAGA AGAAGTTAAA CCTTGGTTAC GTACGGTTTA TGGAAGTCAA
1351  AAAGAATTAG TCACTCCTAC GGTCATTGCC GGTGTCACTT TTTCTGAAAA
1401  ACCAGAAGAA ACACCAAATC CATTGAAACC TTGGGTATCT TTAGAGCATG
1451  ATGGTAGGCC AAAAACCATT AAACCAGAAA TCAACAAAGG TCGAACCAAG
1501  AAGGGAAGAC CTGATTACTC AACTTACTTC AAAACGGTAA GTTCCCACAC
1551  ATATTCTTAT GAAGAATTGA AGGCTCACAA TATGGGCCCT AATGAAGTTT
1601  TTGTAGAAGA AGAGTATATT GATGAAGATG ACACCTACGT CTCCCTGAAT
1651  CCTATTGTCA GATGTACTCC TAATCTTTAC TTCAATAAAG GTCTAGCAAA
1701  GGATATCCGC AGTGAGCCAT TTTGTACCCC TTATGAGAAT TCTAGATGGA
1751  AGGTTGACAA GACTTACTTC GTTACTTGGT ATACAAGATT TTTTACAGAT
1801  GAGAATTCCG GTAAAGTTGC TGATAAGGTT CGTGTTCATT TGTCCTATGT
1851  TAAAGAAAAC CCCGTAGAGA AGGGCAATTA TAAAAGAGAT ATCCCTGCAA
1901  CTTTTTTCTC TTCCGAATGG ATTGATAATG ACAACGGTCT AATGCCGGTT
1951  GAGGTCAGAG ATGAATGGCT GCAGGACCAA TTTGATCGTA GGATCGTTGT
2001  ATCAGTTCAG CCAATATACA TATCAGATGA AGATTTCGAT CCACTACAAT
2051  ACGGTATTTT ATTATACATC ACTAAGGGTT CAAAAGTGTT TAAGCCTACT
2101  AAGGAGCAAC TGGCTTTAGA CGATGCAGGT ATAACAAATG ATCAGTGGTA
2151  TTATGTTGCA TTATCTATCC CTACTGTCGT GGTGGTATTT TTCGTCTTCA
2201  TGTACTTTTT CTTATATGTC AACGGGAAAA ACAGAGATTT CACAGATGTT
2251  ACTAGAAAAG CTTTAAACAA GAAACGCCGT GTTTTGGGTA AGTTCTCGGA
2301  GATGAAGAAA TTCAAAAACA TGAAAAATCA CAAGTACACC GAATTGCCAT
2351  CTTATAAGAA AACCAGTAAA CAAAATTAG
```

FIGURE 57. *YKL077w* Protein Sequence

```
YKL077W  Length: 392  March 26, 1999 16:50  Type: P  Check: 1732  ..

      1  MRFHDSILIF FSLASLYQHV HGARQVVRPK EKMTTSEEVK PWLRTVYGSQ

     51  KELVTPTVIA GVTFSEKPEE TPNPLKPWVS LEHDGRPKTI KPEINKGRTK

    101  KGRPDYSTYF KTVSSHTYSY EELKAHNMGP NEVFVEEEYI DEDDTYVSLN

    151  PIVRCTPNLY FNKGLAKDIR SEPFCTPYEN SRWKVDKTYF VTWYTRFFTD

    201  ENSGKVADKV RVHLSYVKEN PVEKGNYKRD IPATFFSSEW IDNDNGLMPV

    251  EVRDEWLQDQ FDRRIVVSVQ PIYISDEDFD PLQYGILLYI TKGSKVFKPT

    301  KEQLALDDAG ITNDQWYYVA LSIPTVVVVF FVFMYFFLYV NGKNRDFTDV

    351  TRKALNKKRR VLGKFSEMKK FKNMKNHKYT ELPSYKKTSK QN
```

FIGURE 58. *YGR046w* DNA Sequence

Sequence contains 599bp of 5' promoter sequence.
Symbols: 1 to: 1757 from: chr7.gcg   ck: 9962, 584290 to: 586046
Chromosome VII Sequence
Nature 387:81-84 [97313265] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome VII.
Tettelin, H., Agostoni Carbone, M. L., Albermann, K., Albers, M.,
Arroyo, J., Backes, U., Barreiros, T., Bertani, I., Bjourson, A. J., . . .

gcgseq.tmp.228 Length: 1757 March 26, 1999 16:44 Type: N Check: 9449 ..

```
   1  TCTCACTCCG GCGGCCATTT TACGTGACGA AGCATCCCTT ACAACAGAAA
  51  GAAGAAAAAA GATATGCCGC TTTGCGGTTT CTTTCTGGCA ATGTATGCAC
 101  TCATAATGCT ACTCGTTTAC CCACTATCCC TGTCCAAACT AAAGAGGGAG
 151  GAAAGCACTT TTTGCATTTA CACATCGTAG ATTATAAAAT GATCGTTAAC
 201  AGGCGCTTGT GATTTTGAAT TTAAGAAATG TGGACTAGAG AAGTCTTAAA
 251  TCGCCAATGC TGTACCAGAC TCTCTATAGC ATCTAAACAC GAAATTCAAC
 301  TGTTATCTTA GTTTTTCACT TACCAGTAGC GCGCTTGTTA TTCCCACGTT
 351  ATTATTTGCC CCCACATCAT AGGTCAAGTG ACCTTCTCTT ACCCGACATG
 401  AATAAAGAAA AGAAAGAAA TCATACCCTT CAGCCTGTTT AGCCATAAAT
 451  AGTAAAGAGT AGATGTTTCG ACGGACTAAA TAATGTGAAA AAGGTTCTAA
 501  AACCTTCAAA ACAATTAAAC TTGAGAAACG TTGCTATAGG ATTGAGCTAA
 551  TAATTTGAAT TAATAGGAGC TGCTTTTTAC TTTGATATAT CCTGAAGTTA
 601  TGTTACGAGT TTCTGAAAAT GGTCTACGGT TTCTGCTGAA ATGCCATTCA
 651  ACGAACGTAA GCATGTTTAA TAGGCTTCTG AGTACTCAAA TAAAGGAGGG
 701  GAGAAGTTCC ATAGATGATG CTGGCATTAT CCCCGATGGA ACTATTAACG
 751  AAAGGCCGAA TCACTACATC GAGGGAATTA CTAAAGGCAG TGATCTGGAC
 801  CTCTTGGAAA AAGGTATAAG AAAAACTGAC GAAATGACTT CCAATTTTAC
 851  AAATTATATG TACAAGTTTC ACAGATTGCC CCCCAACTAT GGAAGTAACC
 901  AGCTCATTAC TATCGATAAG GAACTTCAAA AGGAACTGGA TGGGGTAATG
 951  TCATCCTTCA AAGCTCCGTG CCGGTTTGTA TTTGGTTACG GCTCAGGAGT
1001  TTTCGAACAA GCGGGATATT CCAAAAGTCA TAGCAAACCT CAAATCGATA
1051  TAATCTTGGG CGTCACATAT CCATCACATT TTCACTCTAT TAATATGAGG
1101  CAGAATCCGC AACATTATTC AAGTTTGAAA TACTTCGGTT CCGAGTTCGT
1151  GTCTAAATTT CAACAGATCG GCGCAGGCGT ATATTTTAAT CCATTTGCAA
1201  ATATAAATGG ACACGACGTA AAATATGGGG TGGTTTCTAT GGAAACACTT
1251  TTAAAGGACA TAGCTACTTG GAATACATTC TATTTAGCAG GACGACTACA
1301  AAAGCCTGTA AAAATATTGA AGAATGATTT GAGAGTGCAA TATTGGAACC
1351  AATTAAACTT AAAAGCTGCA GCTACTTTGG CCAAACATTA CACCTTAGAG
1401  AAAAATAACA ATAAGTTTGA CGAATTCCAA TTTTACAAGG AGATCACTGC
1451  CTTAAGTTAT GCAGGTGATA TTAGATACAA ACTGGGTGGA GAAAATCCCG
1501  ACAAAGTTAA CAACATTGTT ACCAAAAACT TTGAAAGATT TCAAGAGTAT
1551  TACAAGCCGA TTTACAAAGA AGTGGTCCTA AATGATTCAT TTTATCTTCC
1601  AAAAGGGTTC ACATTAAAGA ATACTCAGAG ACTTTTGCTC AGCCGTATTA
1651  GTAAATCAAG TGCATTACAA ACTATTAAAG GTGTTTTCAC AGCTGGAATC
1701  ACAAAGTCAA TTAAGTATGC TTGGGCCAAA AAACTAAAAT CGATGAGGAG
1751  AAGCTAG
```

FIGURE 59. *YGR046w* Protein Sequence

Nature 387:81-84 [97313265] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome VII.

Tettelin, H., Agostoni Carbone, M. L., Albermann, K., Albers, M.,
Arroyo, J., Backes, U., Barreiros, T., Bertani, I., Bjourson, A. J.,
Bruckner, M., Bruschi, C. V., Carignani, G., Castagnoli, L., Cerdan, et al.

YGR046W Length: 385 March 26, 1999 16:46 Type: P Check: 4137 ..

```
  1  MLRVSENGLR FLLKCHSTNV SMFNRLLSTQ IKEGRSSIDD AGIIPDGTIN

 51  ERPNHYIEGI TKGSDLDLLE KGIRKTDEMT SNFTNYMYKF HRLPPNYGSN

101  QLITIDKELQ KELDGVMSSF KAPCRFVFGY GSGVFEQAGY SKSHSKPQID

151  IILGVTYPSH FHSINMRQNP QHYSSLKYFG SEFVSKFQQI GAGVYFNPFA

201  NINGHDVKYG VVSMETLLKD IATWNTFYLA GRLQKPVKIL KNDLRVQYWN

251  QLNLKAAATL AKHYTLEKNN NKFDEFQFYK EITALSYAGD IRYKLGGENP

301  DKVNNIVTKN FERFQEYYKP IYKEVVLNDS FYLPKGFTLK NTQRLLLSRI

351  SKSSALQTIK GVFTAGITKS IKYAWAKKLK SMRRS
```

69/88

FIGURE 60. *YJR041c* DNA Sequence

This sequence includes 1000bp of 5' promoter sequence.
Symbols: 1 to: 4525   from: chr10.gcg /rev   ck: 4711, 509927 to: 514451
Chromosome X Sequence
EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of
Saccharomyces cerevisiae chromosome X.
Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N.,
Chuat, J. C., Coster, F., Cziepluch, C., De Haan, M., Domdey, H., . . .

gcgseq.tmp.25123 Length: 4525 March 26, 1999 11:33 Type: N Check: 4481
  ..

```
   1  TACCTGCTGT AGAATCCTTC ACTGAAAACA CTTGTTCAAT ATATTCTTCA
  51  TCTGGTTCAC CGTCTGATCT ATTAATCCAG TTTAGCAATG ACTCAATAAA
 101  CTCTGATCTG TTCTCCTCTA CATCCTGACC ATCTAATATG AAGTACATTG
 151  TCCTCAGACA GTTTAAAACG GTTAAAGATT CTTCCAACTC ATAAAATCGG
 201  TTCACTCTTC CATCCTGATC CTTGACTCTA CCAATAAACA CTTCCAATTC
 251  ATTCAGAATC GCCTCCATGG CCAGATTTAC TGTTGCATTA TGCTCCTTCG
 301  CGAAATTAGA ATTAACAACT CCAATCGTTG GTACATTAAA CACTCTGTCA
 351  TCACCTAAAT CACGGTAAAT TTCAAATAAA CCTGATACGT ATGCAGAAAA
 401  CTCTTTGCTG GTATCTAATC TAGGAATTCT AACAGGATAA AGCTTATATT
 451  TATCTTTTGC AGTTATGAAT GCCATATTTT GGTAAGAAAG TGGCCCCAGC
 501  TTGAACTTTA AAGGCATCTT GTCGCCATTT TTTTCAATCG GTTGATCATT
 551  TACAGTCATA GGGACCAGGA TAGCCCCGCT GACTGGGTCC CTTTTATATA
 601  GTTGTTCTTC TTCATCGGTC TTGTTATTAC TAAGTTGCGC CGTTCCGTCG
 651  TCCAAAAAAT CAAATTGATC GACGTCCATA AGTAATCGAT TTGAATCATC
 701  GATTGTCATA TCTGATAATT GCGTTCTGGC TCACGCTTAT TGACTCAACT
 751  CAAGACCGTA AGTTCAATGT TTTCTATACA ACTACAATTT GTACAAGGCT
 801  TGACTTCCAT CCAACTAAAA AACCTCTCCG TCGTGCGCGA TCTGAAAAAT
 851  TTCACTTAGC TCATCTCAAA ATGATCGCTA AGAGGGCACT TGGTCACAAC
 901  TACAGAATTG TTTACTAGCA TAGGAACATC TCTGTCTAAG ATTTAGCTTG
 951  CCATCAATTA TCTTTGGAAA AACAGAGAGT ATACTGCACT TTTTGATAAT
1001  ATGGGTGATC TTACAGAAGA ACTATCTATC CCAGACAATG CCCAAGATTT
1051  GTCGAAATTA CTACGTTCGA CGAGCACAAA ACCCCATCAA ATTGCCGAGA
1101  TAGTTTCAAA ATTTGATAAA TTAGAAACCT ACTTTCCAAA AAAAGAAATT
1151  TTCGTCTTAG ATTTACTCAT TGATAGGCTC AACAATGGAA ATTTGGATGA
1201  TTTTAAGACC AGTGAACATA CTTGGATAAT TTTCACGAGA TTATTAGATG
1251  CTATTAATGA TCCAATTTCG ATAAAAAAAC TACTCAAAAA ATTGAAGACT
1301  GTGCCTGTAA TGATAAGAAC ATTTTTCCTT TGGCCTAAAG ACAAATTACT
1351  TACACGTAGC GTTTCGTTTA TAAAAGCATT TTTTGCGATT AATGACTACT
1401  TGATTGTCAA TTTTTCTGTT GAAGAGTCTT TTCAACTTTT AGAACATGCC
1451  ATAAATGGAT TAAGTTCGTG CCCGACGACT GACTTTGCGC TTTCATACTT
1501  GCAAGATGCC TGCAATCTAA CTCATGTTGA CAATATTACT ACAACGGATA
1551  ACAAAATTGC TACTTGTTAC TGCAAGCATA TGCTACTACC AAGTTTAAGA
1601  TATTTCGCAC AGACCAAAAA TTCTGCATCT TCAAACCAAT CCTTCATTCG
1651  TCTATCTCAT TTTATGGGAA AGTTCCTTTT ACAACCACGC ATAGATTACA
1701  TGAAATTAAA TAAAAAGTTT GTCCAAGAGA ATGCGTCCGA AATTACCGAC
1751  GATATGGCTT ATTATTATTT TGCCACTTTC GTCACTTTCT TATCAAAAGA
1801  CAATTTTGCT CAACTAGAAG TCATCTTTAC AATTTTAGGT GCCAAGAAAC
1851  CTAGTTTAGA ATGCAGATTT CTGAATCTTT TATCGGAATC GAAGAAAACC
1901  GTATCTCAAG AGTTCCTTGA AGCATTATTG CTTGAAATGT TAGCGTCGAC
1951  TGATGAATCT GGAGTGTTAT CATTAATACC AATTATCCTT AAATTGGATA
2001  TCGAGGTTGC TATTAAACAT ATTTTTCGGT TACTTGAATT GATTCAGCTC
2051  GAAAATTTGA ACGATCCTCT CTTTTCCTCT CATATTTGGG ATTTAATAAT
2101  CCAATCACAC GCTAACGCAA GGGAATTATC AGATTTTTTT GCCAAAATAA
2151  ATGAGTACTG TTCCAGAAAA GGACCCGATT CCTATTTTTT GATAAATCAT
2201  CCTGCATATG TCAAGTCTAT AACGAAGCAA TTGTTCACTT TATCTTCTTT
2251  ACAATGGAAA AATCTATTGC AAGCTTTACT TGACCAAGTC AATCACGATT
2301  CCACCAACAG GGTTCCTTTA TATTTAATAC GCATATGCTT GGAGGGACTA
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2351  TCAGAGGGCG CATCGCGCGC AACTCTCGAT GAGGTAAAGC CTATTTTATC
2401  TCAAGTATTT ACTTTGGAAT CATTTAATAA CAGTCTTCAA TGGGACCTAA
2451  AGTATCATAT AATGGAAGTC TACGATGATA TTGTCCCTGC AGAGGAACTA
2501  GAAAAAATCG ATTACGTGTT ATCTTCTAAT ATTTTTGATA CTACATCGGC
2551  TGATGTTGAA GAACTGTTCT TTTATTGCTT CAAATTGAGA GAATATATTT
2601  CGTTCGATCT TTCTGATGCA AAAAAAAAAT TCATGAGGCA CTTTGAAATC
2651  CTTGACGAAG AAAGAAAGTC AAACTTATCA TACTCTGTTG TGTCCAAATT
2701  TGCAACATTA GTAAACAACA ACTTTACAAG AGAACAAATT TCTTCTTTAA
2751  TTGATTCATT ACTATTGAAC TCGACAAATT TATCTTCGTT ATTAAAAAAT
2801  GATGACATTT TTGAGGAGAC AAATATCACG TACGCTTTAA TAAACAAGCT
2851  TGCTTCATCA TACCATCAAA CCTTCGCTCT AGAAGCTTTG ATTCAAATTC
2901  CTATCCAATG CATCAACAAA AACGTTAGAG TGGCTCTCAT TAACAATCTA
2951  ACATGCGAAT CATTTTGCCT TGATTCCGCT ACTAGAGAAT GCCTCCTTCA
3001  TTTATTGTCA AGCCCGACCT TCAAGAGCAA CATTGAAACA AATTTCTACG
3051  AATTATGTGA GAAACAATA ATGAGCCCCG AAATGGCCAT TTCAGAGACA
3101  GGTGATGAAA AAAAGGAAAT AGAAGACAAA ATATCTATTT TCGAAAAAGT
3151  TTGGACTAAT CATCTGTCAC AGGCAAAGGA GCCTGTGAGT GAGAAGTTCT
3201  TAGAATCTGG TTACGATATC GTTAAACAGT CAATGTCATT GTCCAATGGT
3251  GATAGCAAAC TAATTATCGC CGGGTTTACT ATCGCAAAT TTTTGAAACC
3301  AGATAACAAG CATAGAGATA TACAAGGTAT GGCAATTAGC TATGCTGTTA
3351  AAATTTTGGA AAACTACTCT GAAAATTTTG AATCTGAAAC AATTCCCCTT
3401  TTCAGAATAT CAATGTCTAC ATTGTACAAG ATTATAACGA CCGGACAAGG
3451  CGATATTTCT AAGCATAAAT CGAGAATTCT GGATATATTT TCCAAAATTA
3501  TGCTTCGATA TCATTCTAAA AAAGTGTACC ATGCGCCAGA AGAACAGGAA
3551  ATGTTTTTGG TTCATTCACT CCTTACAGAA AACAAGTTGG AGTATATTTT
3601  TGCAGAGTAC TTAAATATTG AGCATACAGA TAAGTGCGAT TCTGCCTTGG
3651  GGTTCTGCTT GGAAGAAAGT CTTAAACAAG GTCCTGATGC GTTTAACCGC
3701  CTGCTCTGGA ACAGTGCTAA ATCGTTTTCC ACCATTAGCC AACCTTGTGC
3751  TGAAAAATTT GTGAGAGTTT TTATCATAAT GTCAAAAAGG ATTGCAAGAG
3801  ACAATAACCT TGGTCATCAC CTATTTGTGA TAGCTTTACT TGAAGCCTAC
3851  ACCTATTGTG ATATAGAAAA ATTTGGCTAC AAGTCATACT TGCTACTGTT
3901  CAATGCTATC AAGGAGTTCT TAGTATCGAA ACCATGGCTA TTCAGCCAAT
3951  ACTGTATTGA AATGCTGCTT CCTTTCTGTT TAAAAACTCT CGCTTTTATA
4001  GTAAACCATG AGTCAACGGA TGAAATCAAT GAAGGCTTTA TTAACATCAT
4051  CGAAGTGATA GATCATATGC TATTAGTTCA CAGGTTTAAA TTTTCCAATC
4101  GTCACCATTT GTTTAACTCC GTTCTTTGCC AGATACTAGA AATAATAGCA
4151  ATTCATGATG GTACATTGTG TGCAAATTCA GCAGACGCCG TAGCCAGACT
4201  AATAACGAAC TACTGCGAGC CTTATAATGT ATCAAACGCT CAAAATGGGC
4251  AGAAAAATAA CTTAAGCTCA AAGATAAGTT TGATAAAGCA GTCCATCAGA
4301  AAAAATGTAC TTGTGGTTCT AACGAAATAT ATACAGTTGT CTATTACGAC
4351  GCAGTTCAGT TTAAACATAA AAAAGAGTCT GCAGCCCGGT ATTCATGCGA
4401  TTTTTGATAT ATTATCTCAG AACGAGTTGA ATCAATTGAA CGCTTTCCTT
4451  GACACACCTG GGAAACAATA TTTCAAAGCA CTTTACCTCC AATACAAAAA
4501  GGTTGGTAAA TGGCGCGAAG ATTAA
```

FIGURE 60 (cont).

FIGURE 61.   *YJR041c* Protein Sequence

EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide sequence of
Saccharomyces cerevisiae chromosome X.
Galibert, F., Alexandraki, D., Baur, A., Boles, E., Chalwatzis, N.,
Chuat, J. C., Coster, F., Cziepluch, C., De Haan, M., Domdey, H.,
Durand, P., Entian, K. D., Gatius, M., Goffeau, A., Grivell, L. A., et al.

YJR041C Length: 1174 March 26, 1999 11:35 Type: P Check: 5083 ..

```
   1  MGDLTEELSI PDNAQDLSKL LRSTSTKPHQ IAEIVSKFDK LETYFPKKEI

  51  FVLDLLIDRL NNGNLDDFKT SEHTWIIFTR LLDAINDPIS IKKLLKKLKT

 101  VPVMIRTFFL WPKDKLLTRS VSFIKAFFAI NDYLIVNFSV EESFQLLEHA

 151  INGLSSCPTT DFALSYLQDA CNLTHVDNIT TTDNKIATCY CKHMLLPSLR

 201  YFAQTKNSAS SNQSFIRLSH FMGKFLLQPR IDYMKLNKKF VQENASEITD

 251  DMAYYYFATF VTFLSKDNFA QLEVIFTILG AKKPSLECRF LNLLSESKKT

 301  VSQEFLEALL LEMLASTDES GVLSLIPIIL KLDIEVAIKH IFRLLELIQL

 351  ENLNDPLFSS HIWDLIIQSH ANARELSDFF AKINEYCSRK GPDSYFLINH

 401  PAYVKSITKQ LFTLSSLQWK NLLQALLDQV NHDSTNRVPL YLIRICLEGL

 451  SEGASRATLD EVKPILSQVF TLESFNNSLQ WDLKYHIMEV YDDIVPAEEL

 501  EKIDYVLSSN IFDTTSADVE ELFFYCFKLR EYISFDLSDA KKKFMRHFEI

 551  LDEERKSNLS YSVVSKFATL VNNNFTREQI SSLIDSLLLN STNLSSLLKN

 601  DDIFEETNIT YALINKLASS YHQTFALEAL IQIPIQCINK NVRVALINNL

 651  TCESFCLDSA TRECLLHLLS SPTFKSNIET NFYELCEKTI MSPEMAISET

 701  GDEKKEIEDK ISIFEKVWTN HLSQAKEPVS EKFLESGYDI VKQSMSLSNG

 751  DSKLIIAGFT IAKFLKPDNK HRDIQGMAIS YAVKILENYS ENFESETIPL

 801  FRISMSTLYK IITTGQGDIS KHKSRILDIF SKIMLRYHSK KVYHAPEEQE

 851  MFLVHSLLTE NKLEYIFAEY LNIEHTDKCD SALGFCLEES LKQGPDAFNR

 901  LLWNSAKSFS TISQPCAEKF VRVFIIMSKR IARDNNLGHH LFVIALLEAY

 951  TYCDIEKFGY KSYLLLFNAI KEFLVSKPWL FSQYCIEMLL PFCLKTLAFI

1001  VNHESTDEIN EGFINIIEVI DHMLLVHRFK FSNRHHLFNS VLCQILEIIA

1051  IHDGTLCANS ADAVARLITN YCEPYNVSNA QNGQKNNLSS KISLIKQSIR

1101  KNVLVVLTKY IQLSITTQFS LNIKKSLQPG IHAIFDILSQ NELNQLNAFL

1151  DTPGKQYFKA LYLQYKKVGK WRED
```

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FIGURE 62. *HES1* DNA Sequence

DNA sequence includes 1089bp 5' promoter sequence.
Symbols: 1 to: 2394 from: chr15.gcg   ck: 9129, 780903 to: 783296
Chromosome XV Sequence
Nature 387:98-102 [97313270] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XV.
Dujon, B., Albermann, K., Aldea, M., Alexandraki, D., Ansorge, W.,
Arino, J., Benes, V., Bohn, C., Bolotin-Fukuhara, M., Bordonne, R., . . .

gcgseq.tmp.10515 Length: 2394 March 26, 1999 14:35 Type: N Check: 4842
..

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   1   CATGGCTGGA GGAAAGATTC CTATTGTAGG AATTGTGGCA TGTTTACAGC
  51   CGGAGATGGG GATAGGATTT CGTGGAGGTC TACCATGGAG GTTGCCCAGT
 101   GAAATGAAGT ATTTCAGACA GGTCACTTCA TTGACGAAAG ATCCAAACAA
 151   AAAAAATGCT TTGATAATGG GAAGGAAGAC ATGGGAATCC ATACCGCCCA
 201   AGTTTCGCCC ACTGCCCAAT AGAATGAATG TCATTATATC GAGAAGCTTC
 251   AAGGACGATT TTGTCCACGA TAAAGAGAGA TCAATAGTCC AAAGTAATTC
 301   ATTGGCAAAC GCAATAATGA ACCTAGAAAG CAATTTTAAG GAGCATCTGG
 351   AAAGAATCTA CGTGATTGGG GGTGGCGAAG TTTATAGTCA AATCTTCTCC
 401   ATTACAGATC ATTGGCTCAT CACGAAAATA AATCCATTAG ATAAAAACGC
 451   AACTCCTGCA ATGGACACTT TCCTTGATGC GAAGAAATTG GAAGAAGTAT
 501   TTAGCGAGCA AGATCCGGCC CAGCTGAAAG AATTTCTTCC CCCTAAAGTA
 551   GAGTTGCCCG AAACAGACTG TGATCAACGC TACTCGCTGG AAGAAAAAGG
 601   TTATTGCTTC GAATTCACTC TATACAATCG TAAATGAAAC CTCTCCGCCC
 651   GTATATTTTT TTTAATATGT TAAATAGTGA TAGAACTGAT AAGCCTCATT
 701   TTCTTTTATT GGGCTCCAAG ACGCGAACTG TTCGTAGGGT AACCGTTTGA
 751   CACCTAAACG ACCTTTCAGC CTCACCTGCA GTATTTCTTC AACAACGCCT
 801   GTCGCTATGT TAAATAATAG CAATCGTTTG TGATCACCAT TGTCGAATTT
 851   GACGCGCTTA AACAAAAACC ATTGTTTTGG CCTCGTTCCC TGCATTCAAC
 901   AAAAGAGCAA GGTATGCCGT CAAACAGTCG TTAAAAGAGA AGGTTTATAA
 951   ACTATCTTGT TTTGTACTTT GCTGTCCCGG ATCCAGTTGG GTCTTCTTTT
1001   CAACCTGTCT GAGTCCGATC TTTCTTTCCC TACTTGAAGC TCCATATATC
1051   TAAGTCATCT AAGTGTATCC TGCTAGATTA CAAACGAAAA TGTCTCAACA
1101   CGCAAGCTCA TCTTCTTGGA CTTCTTTTTT GAAATCGATA AGTTCGTTCA
1151   ACGGAGATCT ATCGTCTTTG TCTGCACCAC CGTTTATTCT TTCTCCCACT
1201   TCCTTAACAG AGTTTTCTCA GTATTGGGCT GAACATCCAG CTTTATTTCT
1251   GGAGCCTTCG TTGATTGATG GTGAAAACTA CAAAGATCAC TGTCCCTTTG
1301   ACCCAAATGT GGAATCAAAG GAAGTGGCGC AGATGTTGGC GGTTGTTAGG
1351   TGGTTTATTT CTACTTTGAG ATCTCAATAC TGCTCTAGAA GCGAATCGAT
1401   GGGTTCTGAA AAGAAGCCTT TGAACCCATT CTTGGGTGAG GTATTTGTTG
1451   GAAAGTGGAA AAATGATGAG CATCCAGAGT TTGGTGAAAC GGTTCTTTTA
1501   AGTGAGCAAG TTTCACATCA TCCACCTATG ACAGCATTTT CGATTTTTAA
1551   TGAAAAAAAT GATGTTTCTG TTCAAGGATA CAATCAAATT AAAAACTGGTT
1601   TTACCAAAAC ATTGACGCTA ACGGTCAAAC CATACGGGCA TGTCATTTTG
1651   AAGATTAAAG ATGAGACCTA CCTGATTACA ACCCCGCCTT TGCATATCGA
1701   AGGTATTTTA GTCGCTTCTC CATTTGTTGA ATTAGGAGGC AGGTCATTCA
1751   TACAGTCATC AAATGGTATG TTATGTGTTA TAGAATTTTC AGGAAGGGGG
1801   TATTTCACAG GGAAGAAGAA CTCCTTTAAG GCAAGAATTT ACAGAAGCCC
1851   ACAAGAGCAT AGTCATAAAG AAAATGCGCT ATACCTAATC TCTGGCCAAT
1901   GGTCAGGTGT TTCAACAATT ATAAAAAAAG ACTCGCAAGT TTCACATCAG
1951   TTTTACGATT CATCGGAAAC TCCTACTGAA CATTTATTAG TTAAGCCAAT
2001   CGAAGAACAA CATCCTCTGG AAAGTAGGAG GGCATGGAAG GATGTGGCAG
2051   AAGCAATCAG ACAAGGAAAT ATTAGTATGA TAAAAAAGAC TAAGGAAGAA
2101   CTAGAAAATA AGCAAAGAGC CTTGAGAGAA CAAGAACGCG TAAAAGGTGT
2151   GGAATGGCAA AGAAGATGGT TCAAACAAGT GGACTACATG AATGAAAATA
2201   CATCAAATGA TGTAGAGAAA GCAAGTGAAG ATGATGCCTT TAGGAAATTG
2251   GCGTCCAAAC TGCAGCTTTC TGTGAAAAAT GTGCCAAGTG GGACATTGAT
2301   TGGCGGCAAA GATGATAAGA AAGATGTTTC AACCGCATTG CATTGGAGGT
```

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FIGURE 63.   *HES1* Protein Sequence

Nature 387:98-102 [97313270] (1997) The nucleotide sequence of
Saccharomyces cerevisiae chromosome XV.

Dujon, B., Albermann, K., Aldea, M., Alexandraki, D., Ansorge, W.,
Arino, J., Benes, V., Bohn, C., Bolotin-Fukuhara, M., Bordonne, R.,
Boyer, J., Camasses, A., Casamayor, A., Casas, C., Cheret, G., et al.

```
  YOR237W  Length: 434  March 26, 1999 14:37  Type: P  Check: 7501   ..

      1  MSQHASSSSW TSFLKSISSF NGDLSSLSAP PFILSPTSLT EFSQYWAEHP

     51  ALFLEPSLID GENYKDHCPF DPNVESKEVA QMLAVVRWFI STLRSQYCSR

    101  SESMGSEKKP LNPFLGEVFV GKWKNDEHPE FGETVLLSEQ VSHHPPMTAF

    151  SIFNEKNDVS VQGYNQIKTG FTKTLTLTVK PYGHVILKIK DETYLITTPP

    201  LHIEGILVAS PFVELGGRSF IQSSNGMLCV IEFSGRGYFT GKKNSFKARI

    251  YRSPQEHSHK ENALYLISGQ WSGVSTIIKK DSQVSHQFYD SSETPTEHLL

    301  VKPIEEQHPL ESRRAWKDVA EAIRQGNISM IKKTKEELEN KQRALREQER

    351  VKGVEWQRRW FKQVDYMNEN TSNDVEKASE DDAFRKLASK LQLSVKNVPS

    401  GTLIGGKDDK KDVSTALHWR FDKNLWMREN EITI
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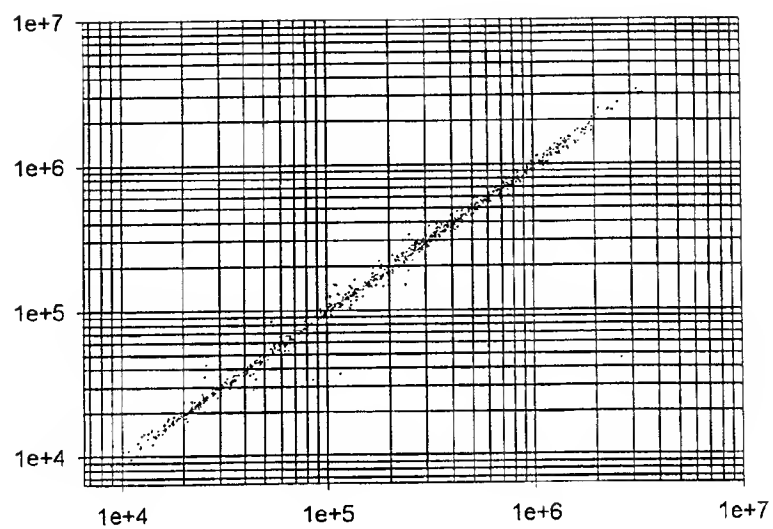
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Figure 64

FIGURE 65.   Rat Gene with Similarity to *YLR100w*

```
LOCUS         1397235          334 aa                          04-FEB-1999
DEFINITION    ovarian-specific protein.
ACCESSION     1397235
PID           g1397235
DBSOURCE      locus RNU44803 accession U448031
KEYWORDS      .
SOURCE        Norway rat.
  ORGANISM    Rattus norvegicus
              Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
              Vertebrata; Eutheria; Rodentia; Sciurognathi; Myomorpha; Muridae;
              Murinae; Rattus.
REFERENCE     1  (residues 1 to 334)
  AUTHORS     Duan,W.R., Linzer,D.I.H. and Gibori,G.
  TITLE       Cloning and characterization of an ovarian-specific protein that
              associates with the short form of the prolactin receptor
  JOURNAL     J. Biol. Chem. 271 (26), 15602-15607 (1996)
  MEDLINE     96279080
REFERENCE     2  (residues 1 to 334)
  AUTHORS     Gibori,G. and Duan,W.R.
  TITLE       Direct Submission
  JOURNAL     Submitted (05-JAN-1996) Geula Gibori, Department of Physiology,
              University of Illinois at Chicago, Chicago, IL 60612, USA
COMMENT       Method: conceptual translation.
FEATURES              Location/Qualifiers
     source           1..334
                      /organism="Rattus norvegicus"
                      /strain="Sprague-Dawley"
                      /db_xref="taxon:10116"
                      /sex="female"
                      /tissue_type="corpus luteum"
                      /dev_stage="pregnant"
                      /cell_type="luteal"
     Protein          1..334
                      /product="ovarian-specific protein"
     CDS              1..334
                      /note="The protein can associate with the short form of
                      prolactin receptor in the rat corpus luteum."
                      /coded_by="U44803:15..1019"
ORIGIN
        1 mrkvvlitga ssgiglalcg rllaedddlh lclacrnlsk agavrdalla shpsaevsiv
       61 qmdvsnlqsv vrgaeevkrr fqrldylyln agimpnpqln lkaffcgifs rnvihmfsta
      121 eglltqndki tadgfqevfe tnlfghfili releplichs dnpsqliwts srnakksnfs
      181 lediqhakgq epyssskyat dllnvalnrn fnqkglyssv tcpgvvmtnl tygilppfvw
      241 tlllpviwll rffahaftvt pyngaealvw lfhqkpesln pltkylsgtt glgtnyvkgq
      301 kmdvdedtae kfyktllele kqvritiqks dhhs
//
```

FIGURE 66. *DAK1* DNA Sequence

This sequence contains 1200bp of 5' promoter sequence.
Symbols: 1 to: 2955   from: chr13.gcg   ck: 8335,
132275 to: 135229

Chromosome XIII Sequence
Nature 387:90-93 [97313268] (1997) The nucleotide sequence
of Saccharomyces cerevisiae chromosome XIII.
Bowman, S., Churcher, C., Badcock, K., Brown, D.,
Chillingworth, T., Connor, R., Dedman, K., Devlin, K.,
Gentles, S., Hamlin, N., Hunt, S., .

gcgseq.tmp.16080 Length: 2955 March 31, 1999 09:57 Type:
N Check: 5254 ..

```
    1   TAATATAAAT ACTAGTCGTT AGATGATAGT TGCTTCTTAT TCCGAAAATG
   51   AGTATGGAAG TGTTGCATAT GATAGGGCGG CTACAGTGAT GGTAAACATA
  101   AGATACTTTA GCGGGAAATT AGCAACTGGA AGTTAAATTA TCTAGACATA
  151   AGTGTGGCGG TCACGCTGAA CGCAGGAGAT CGGATAGATT GATAAGCTGA
  201   TCAAGAACAT TGATCGGTTT GTTGTTTAAA GAATGGTTTT TGAAAACGTT
  251   TGACCAGTTG CTTCTCCCAG ACGCTTACCG ATATGATGAT AAAGATAATA
  301   TCTTCAATTG AATACCCCGT GGATCAGCAC GAATAACAGA AAAAAAGGGT
  351   GAAATTCACC GTAAGCATGA TACGCACTAC GTTCTTCTTA CCTTTGCCAA
  401   CGTGTTGTCT TTGACGTACG TAATTATGGG AGATCGTTGA TGATTAGCCC
  451   CAGCTCACTT TCTTCTTAAT GACTGACCCG CTACTATCAA AATTAAGGTG
  501   TCAAATATCA TGATGAATGA GGTCTCTAGG CGACTCAATT ATACATCTTT
  551   TAGAGATTTT TTTACTACTT GCAGATAATT TCTCAAGGGA TTAGATTCAA
  601   ATCTGGCTTG TCAATTACGC CCTTTTCAAG CTCATCAAAT TGCGTATGTC
  651   ATTCATGCTT CCATTAGGAA CCATAGAAGC ATGGCTGAAA TGGCAATATA
  701   CGGCTTCCCA ATTTCAACTC TAAAGTAATG GCGGTCGAAT TTAATCTATA
  751   TTTTACAGTT TTATACGTAC TTTAAAAGCA ATCAGTAAAC ACCTCTGGTG
  801   CTATTCAAGG GTTTTTTGCC TTTATTTGTT ACTGTCAATT GTCTGGCGCT
  851   GTGATAAAAA ACAAGGCATA AAGCTCCCCC GTCATGAACA TTAAGACTCG
  901   CTAGACGAGA GAGTGAAATA TAATGCATTT CCTGATTTAA ATGCGCTACA
  951   AACATGGTGT AAATCTGGCC CGGAGTGAGT GCTTGCCAAT TTGGCTTCTA
 1001   AGGGAGAAAG ATCAAACCAC TCCCAATTGC GTCATTTTGA AAGAGTGGCC
 1051   ACCTCGCGAG CGTCTGTCGA ACTAACTGAT GAATAAATAT ATAAGGAGAA
 1101   AATCACTTCA ACTTCGCTAC AAGTAGTCAC TATTTGTAGC AACTGTAAAC
 1151   GAACACATCA AAGAATAAGA TTACATTCTA TATCTAAGAC TAAATTTTAA
 1201   ATGTCCGCTA AATCGTTTGA AGTCACAGAT CCAGTCAATT CAAGTCTCAA
 1251   AGGGTTTGCC CTTGCTAACC CCTCCATTAC GCTGGTCCCT GAAGAAAAAA
 1301   TTCTCTTCAG AAAGACCGAT TCCGACAAGA TCGCATTAAT TTCTGGTGGT
 1351   GGTAGTGGAC ATGAACCTAC ACACGCCGGT TTCATTGGTA AGGGTATGTT
 1401   GAGTGGCGCC GTGGTTGGCG AAATTTTTGC ATCCCCTTCA ACAAAACAGA
 1451   TTTTAAATGC AATCCGTTTA GTCAATGAAA ATGCGTCTGG CGTTTTATTG
 1501   ATTGTGAAGA ACTACACAGG TGATGTTTTG CATTTTGGTC TGTCCGCTGA
 1551   GAGAGCAAGA GCCTTGGGTA TTAACTGCCG CGTTGCTGTC ATAGGTGATG
 1601   ATGTTGCAGT TGGCAGAGAA AAGGGTGGTA TGGTTGGTAG AAGAGCATTG
 1651   GCAGGTACCG TTTTGGTTCA TAAGATTGTA GGTGCCTTCG CAGAAGAATA
 1701   TTCTAGTAAG TATGGCTTAG ACGGTACAGC TAAAGTGGCT AAAATTATCA
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1751  ACGACAATTT  GGTGACCATT  GGATCTTCTT  TAGACCATTG  TAAAGTTCCT
1801  GGCAGGAAAT  TCGAAAGTGA  ATTAAACGAA  AAACAAATGG  AATTGGGTAT
1851  GGGTATTCAT  AACGAACCTG  GTGTGAAAGT  TTTAGACCCT  ATTCCTTCTA
1901  CCGAAGACTT  GATCTCCAAG  TATATGCTAC  CAAAACTATT  GGATCCAAAC
1951  GATAAGGATA  GAGCTTTTGT  AAAGTTTGAT  GAAGATGATG  AAGTTGTCTT
2001  GTTAGTTAAC  AATCTCGGCG  GTGTTTCTAA  TTTTGTTATT  AGTTCTATCA
2051  CTTCCAAAAC  TACGGATTTC  TTAAAGGAAA  ATTACAACAT  AACCCCGGTT
2101  CAAACAATTG  CTGGCACATT  GATGACCTCC  TTCAATGGTA  ATGGGTTCAG
2151  TATCACATTA  CTAAACGCCA  CTAAGGCTAC  AAAGGCTTTG  CAATCTGATT
2201  TTGAGGAGAT  CAAATCAGTA  CTAGACTTGT  TGAACGCATT  TACGAACGCA
2251  CCGGGCTGGC  CAATTGCAGA  TTTTGAAAAG  ACTTCTGCCC  CATCTGTTAA
2301  CGATGACTTG  TTACATAATG  AAGTAACAGC  AAAGGCCGTC  GGTACCTATG
2351  ACTTTGACAA  GTTTGCTGAG  TGGATGAAGA  GTGGTGCTGA  ACAAGTTATC
2401  AAGAGCGAAC  CGCACATTAC  GGAACTAGAC  AATCAAGTTG  GTGATGGTGA
2451  TTGTGGTTAC  ACTTTAGTGG  CAGGAGTTAA  AGGCATCACC  GAAAACCTTG
2501  ACAAGCTGTC  GAAGGACTCA  TTATCTCAGG  CGGTTGCCCA  AATTTCAGAT
2551  TTCATTGAAG  GCTCAATGGG  AGGTACTTCT  GGTGGTTTAT  ATTCTATTCT
2601  TTTGTCGGGT  TTTTCACACG  GATTAATTCA  GGTTTGTAAA  TCAAAGGATG
2651  AACCCGTCAC  TAAGGAAATT  GTGGCTAAGT  CACTCGGAAT  TGCATTGGAT
2701  ACTTTATACA  AATATACAAA  GGCAAGGAAG  GGATCATCCA  CCATGATTGA
2751  TGCTTTAGAA  CCATTCGTTA  AAGAATTTAC  TGCATCTAAG  GATTTCAATA
2801  AGGCGGTAAA  AGCTGCAGAG  GAAGGTGCTA  AATCCACTGC  TACATTCGAG
2851  GCCAAATTTG  GCAGAGCTTC  GTATGTCGGC  GATTCATCTC  AAGTAGAAGA
2901  TCCTGGTGCA  GTAGGCCTAT  GTGAGTTTTT  GAAGGGGGTT  CAAAGCGCCT
2951  TGTAA
```

FIGURE 66 (cont).

FIGURE 67. *DAK1* Protein Sequence

Nature 387:90-93 [97313268] (1997) The nucleotide sequence
of Saccharomyces cerevisiae chromosome XIII.

Bowman, S., Churcher, C., Badcock, K., Brown, D.,
Chillingworth, T., Connor, R., Dedman, K., Devlin, K.,
Gentles, S., Hamlin, N., Hunt, S., Jagels, K., Lye, G.,
Moule, S., Odell, C., Pearson, D., Rajandream, et al.

YML070W Length: 584 March 31, 1999 09:58 Type: P Check:
167 ..

```
   1  MSAKSFEVTD PVNSSLKGFA LANPSITLVP EEKILFRKTD SDKIALISGG
  51  GSGHEPTHAG FIGKGMLSGA VVGEIFASPS TKQILNAIRL VNENASGVLL
 101  IVKNYTGDVL HFGLSAERAR ALGINCRVAV IGDDVAVGRE KGGMVGRRAL
 151  AGTVLVHKIV GAFAEEYSSK YGLDGTAKVA KIINDNLVTI GSSLDHCKVP
 201  GRKFESELNE KQMELGMGIH NEPGVKVLDP IPSTEDLISK YMLPKLLDPN
 251  DKDRAFVKFD EDDEVVLLVN NLGGVSNFVI SSITSKTTDF LKENYNITPV
 301  QTIAGTLMTS FNGNGFSITL LNATKATKAL QSDFEEIKSV LDLLNAFTNA
 351  PGWPIADFEK TSAPSVNDDL LHNEVTAKAV GTYDFDKFAE WMKSGAEQVI
 401  KSEPHITELD NQVGDGDCGY TLVAGVKGIT ENLDKLSKDS LSQAVAQISD
 451  FIEGSMGGTS GGLYSILLSG FSHGLIQVCK SKDEPVTKEI VAKSLGIALD
 501  TLYKYTKARK GSSTMIDALE PFVKEFTASK DFNKAVKAAE EGAKSTATFE
 551  AKFGRASYVG DSSQVEDPGA VGLCEFLKGV QSAL
```

FIGURE 68. *PGU1* DNA Sequence

DNA sequence includes 1200bp of 5' promoter sequence.
Symbols: 1 to: 2286 from: chr10.gcg   ck: 4711,
721304 to: 723589
Chromosome X Sequence
EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide
sequence of Saccharomyces cerevisiae chromosome X.
Galibert, F., Alexandraki, D., Baur, A., Boles, E.,
Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De
Haan, M., Domdey, H., . . .

gcgseq.tmp.30022 Length: 2286 March 31, 1999 09:20 Type:
N Check: 4618 ..

```
   1  ATGATTCTGA CGACCCTTTG ATAGTGGCAA TGATCAAAAA GAAAAAAAAA
  51  AGATAAGACG GTAGTGTGAA GATGACATAT AGCGCTACTC TATACTCGTC
 101  CAACTTCGAA AATAATATGT GGTCGTTGGT ACGTTCAGAT AAGAGAATAC
 151  ATCTCGCGCG TACGCATAAT TGTGGTCTAA AAAACCGCTG AAATTTTCTC
 201  AATACTGAAT AGAATCACGC TACTACGACA AGACTCGGTT ACTGTGCCTA
 251  AAATAATCCT GTGATAAACG AGTTATGTTA AACGCAGTAC AGGGGTTAAA
 301  GGGCATTGAG TTTTTGTGAG TGGAAATGCC CCCGTTATAG CTTCCAGTTT
 351  AATTACAAAT TATCAATTTA AGCAAATATA ACTGGAGGAT TGGGGAGGCG
 401  ACTAAAAATG GCTACCACGC TATTAGACAT ACAACATTGA GTATTTTATG
 451  TAATTTTGTT ACTGCTAGCA CGGCCATGCA ATTGGCAACT GAAAGCTATC
 501  TGACAACTTA AATGATTCTT AAAACAATGA CGACTATAAT CTTCTCTAAG
 551  AAGTTTCATA TCCATCTTCC TCATTATTCA GTTTCTTTTT CCTCTTGAAA
 601  GTATCGTAAA GAACAACGTC TTCACATTAG CTATTAGAAG ACCATTGAAC
 651  TACCGGATAT GAGTAAGAGT GATCTTGCCG GAGAGATAAT AGCTGCACAA
 701  AGGCCAAGGA TTAGATTAAT GGGTGCATTG TACGAAAAAA AATAGTTTAC
 751  AGTCATTTAT TCGCAATAAA TCAATTTTTT TTTCAAAAAA TATGTAAGTC
 801  TGATAAAAAA TTCTTCACTG AAGAGAGATG CTTACATTCT AATTCTTGAA
 851  TAAAAGACTC TCTAACGCTG TGAATTCTCT TTAGCTGTAA CGGAAACAGA
 901  GAGTTATTCC GTAGTCACTG AATTTTTTTT TTTTGACGCT ATTATTTAAA
 951  ACCTAGGATA TCCGTCCCAT ACAAACGGC CACGAGTTTC AATCCCAGAA
1001  TGTACGAGTT ATAATTCTCC TAGATGCATG ATACTCGTGC ATTCGTTTAA
1051  CAATCATACC AATTTCCCAT TTTCGGGATA TTAAACATGA ACATACTTTT
1101  TTACTGTGAG AATGTGGTTT CACAATTATT CCATACAGGT ATAAAAACGC
1151  ACAGAACTTC AAACGGGAAG ACTATCTACC CACATTGATG GACAAACGCA
1201  ATGATTTCTG CTAATTCATT ACTTATTTCC ACTTTGTGCG CTTTTGCGAT
1251  CGCAACACCT TTGTCAAAAA GAGATTCCTG TACCCTAACA GGATCTTCTT
1301  TGTCTTCACT CTCAACCGTG AAAAAATGTA GCAGCATCGT TATTAAAGAC
1351  TTAACTGTCC CAGCTGGACA GACTTTAGAT TTAACTGGGT TAAGCAGTGG
1401  TACTACTGTT ACGTTTGAAG GCACAACCAC ATTTCAGTAC AAGGAATGGA
1451  GCGGCCCTTT AATTTCAATC TCAGGGTCTA AAATCAGCGT TGTTGGTGCT
1501  TCGGGACATA CCATTGATGG TCAAGGAGCA AAATGGTGGG ATGGCTTAGG
1551  TGATAGCGGT AAAGTCAAAC CGAAGTTTGT AAAGTTGGCG TTGACGGGAA
1601  CATCTAAGGT CACCGGATTG AATATTAAAA ATGCTCCACA CCAAGTCTTC
1651  AGCATCAATA AATGTTCAGA TTTAACCATC AGCGACATAA CAATTGATAT
1701  CAGAGACGGT GATTCGGCTG GTGGTCATAA TACGGATGGG TTTGATGTTG
1751  GTAGTTCTAG TAACGTCTTA ATTCAAGGAT GTACTGTTTA TAATCAGGAT
1801  GACTGTATTG CTGTGAATTC CGGTTCAACT ATTAAATTTA TGAACAACTA
```

```
1851   CTGCTACAAT GGCCATGGTA TTTCTGTAGG TTCTGTTGGT GGCCGTTCTG
1901   ATAATACAGT CAATGGTTTC TGGGCTGAAA ATAACCATGT TATCAACTCT
1951   GACAACGGGT TGAGAATAAA AACCGTAGAA GGTGCGACAG GCACAGTCAC
2001   TAATGTCAAC TTTATCAGTA ATAAAATTAG CGGCATAAAA AGTTATGGTA
2051   TTGTTATCGA AGGCGATTAT TTGAATAGTA AGACTACTGG AACTGCTACA
2101   GGTGGCGTTC CCATTTCGAA TTTAGTAATG AAGGATATCA CCGGGAGCGT
2151   GAACTCCACA GCGAAGAGGG TTAAAATTTT GGTGAAAAAC GCTACTAACT
2201   GGCAATGGTC TGGGGTGTCA ATTACCGGTG GTTCTTCCTA TTCTGGATGT
2251   TCTGGAATCC CATCTGGATC TGGTGCAAGC TGTTAA
```

FIGURE 68 (cont).

FIGURE 69. *PGU1* Protein Sequence

EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide
sequence of Saccharomyces cerevisiae chromosome X.

Galibert, F., Alexandraki, D., Baur, A., Boles, E.,
Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De
Haan, M., Domdey, H., Durand, P., Entian, K. D., Gatius, M.,
Goffeau, A., Grivell, L. A., et al.

YJR153W Length: 361 March 31, 1999 09:55 Type: P Check:
9795 ..

```
   1  MISANSLLIS  TLCAFAIATP  LSKRDSCTLT  GSSLSSLSTV  KKCSSIVIKD
  51  LTVPAGQTLD  LTGLSSGTTV  TFEGTTTFQY  KEWSGPLISI  SGSKISVVGA
 101  SGHTIDGQGA  KWWDGLGDSG  KVKPKFVKLA  LTGTSKVTGL  NIKNAPHQVF
 151  SINKCSDLTI  SDITIDIRDG  DSAGGHNTDG  FDVGSSSNVL  IQGCTVYNQD
 201  DCIAVNSGST  IKFMNNYCYN  GHGISVGSVG  GRSDNTVNGF  WAENNHVINS
 251  DNGLRIKTVE  GATGTVTNVN  FISNKISGIK  SYGIVIEGDY  LNSKTTGTAT
 301  GGVPISNLVM  KDITGSVNST  AKRVKILVKN  ATNWQWSGVS  ITGGSSYSGC
 351  SGIPSGSGAS  C
```

FIGURE 70. *STE18* DNA Sequence

This sequence contains 600bp of 5' promoter sequence.
Symbols: 1 to: 933   from: chr10.gcg   ck: 4711,
585156 to: 586088

Chromosome X Sequence
EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide
sequence of Saccharomyces cerevisiae chromosome X.
Galibert, F., Alexandraki, D., Baur, A., Boles, E.,
Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De
Haan, M., Domdey, H., . . .

gcgseq.tmp.6719 Length: 933 March 31, 1999 10:01 Type: N
Check: 8833 ..

```
   1 TTCGTTTCTG TCTTGTCTCC CGCTGTTACC TAATAACTTC ATGTGATCTG
  51 CTCCCCCTTC TCGTTAAATA CCACCTTTTC ATCAACCCCG TAGGGCGCGA
 101 CACGTCTAAA ATATTAACCT CTGAATACTT ATTGGGTCAA AATGAATGTT
 151 GATAACTTTC CTTTACAAAA AAAAAACTAA TAGAGTATAT GCATTTCGGT
 201 AGTGAAATAT TCGTTAATGC TAATATGCTC AGTAGTGATC CTAGATTACC
 251 AGTTTTACTG CAGCCATCGT ACAATTTTGG AACGAGTATA AAGAGAGAAA
 301 TTAAAAACGA CAAGAAATAT TCGTACTAGC TTCTCTTCCG GCTTGATGAC
 351 AGTCTTAATA TCATCTGCAA CTCTTGAAAT CTTGCTTTAT AGTCAAAATT
 401 TACGTACGCT TTTCACTATA TAATATGATT TGTCAATGTG ATGAGTGAAT
 451 GTCTCCCTGT TACCCGGTTT TCATGTTGAT TTTTGTTTCA GGCTCTAAAT
 501 GTTTGATGCA ATATTTAACA AGGAGAACAG AAATGTTTTG TGACAGCACC
 551 TGTCAATTTT AGGATAGTAG CAATCGCAAA CGTTCTCAAT AATTCTAAGA
 601 ATGACATCAG TTCAAAACTC TCCACGCTTA CAACAACCTC AGGAACAGCA
 651 ACAGCAACAG CAACAGCTTT CCTTAAAGAT AAAACAATTG AAGTTAAAAA
 701 GAATCAACGA ACTTAACAAT AAACTGAGGA AAGAACTCAG CCGTGAAAGA
 751 ATTACTGCTT CAAATGCATG TCTTACAATA ATAAACTATA CCTCGAATAC
 801 AAAAGATTAT ACATTACCAG AACTATGGGG CTACCCCGTA GCAGGATCAA
 851 ATCATTTTAT AGAGGGTTTG AAAAATGCTC AAAAAAATAG CCAAATGTCA
 901 AACTCAAATA GTGTTTGTTG TACGCTTATG TAA
```

FIGURE 71. *STE18* Protein Sequence

EMBO J. 15:2031-2049 [8641269] (1996) Complete nucleotide
sequence of Saccharomyces cerevisiae chromosome X.

Galibert, F., Alexandraki, D., Baur, A., Boles, E.,
Chalwatzis, N., Chuat, J. C., Coster, F., Cziepluch, C., De
Haan, M., Domdey, H., Durand, P., Entian, K. D., Gatius, M.,
Goffeau, A., Grivell, L. A., et al.

YJR086W Length: 110 March 31, 1999 10:02 Type: P Check:
6859 ..

```
  1   MTSVQNSPRL QQPQEQQQQQ QQLSLKIKQL KLKRINELNN KLRKELSRER
 51   ITASNACLTI INYTSNTKDY TLPELWGYPV AGSNHFIEGL KNAQKNSQMS
101   NSNSVCCTLM
```

FIGURE 72. *YGL198w* DNA Sequence

This sequence contains 989bp of 5' promoter sequence.
Symbols: 1 to: 1775   from: chr7.gcg   ck: 9962,
122605 to: 124379

Chromosome VII Sequence
Nature 387:81-84 [97313265] (1997) The nucleotide sequence
of Saccharomyces cerevisiae chromosome VII.
Tettelin, H., Agostoni Carbone, M. L., Albermann, K.,
Albers, M., Arroyo, J., Backes, U., Barreiros, T., Bertani,
I., Bjourson, A. J., . .

gcgseq.tmp.32650 Length: 1775 March 31, 1999 10:03 Type:
N Check: 2850 ..

```
    1  GAGAATTATT CGCGACTTCA GGTTATCCAA TCGTGTATGT AATCGTATGT
   51  AGGCAAAAGT AAATAGATAT GAACTACATT TTCCTGCTTT ACTTAGACTA
  101  GAGATGTGAC CTCAAAGAAT CTTCTCAAGT AGTATATCTG GAAAAGAGAG
  151  TTTGCAATAA CGACGCCCAA TTGGAAGATG GACCACCATT TAACACGATC
  201  GTTGGTCGAC TCTGCAGTAT TTCTATGCGT CCTTTCTCTA ATAACAATAT
  251  AACTTTGTTC GTCCTTGACT TCCCTGGTTA ATTTGGACAA CTTTCTGACA
  301  GCACTATCCA ATGTATTGGT GTTTGGGTCG TCCAAATCCA CATATACCAC
  351  CCCATGAATG TTGAAAGTCA CGTCTTTTGT CTCGATACCG GTGTTCTCGT
  401  TCAAGAAACA GTATTGGAAA TGTCCCTTGT ATGGAGCAGA CAATGTGATT
  451  TCACCGTGCG ACGTGTCCCT AACCGTTTTC AAAACTTCAT GTCTTTCCGG
  501  CCCGTAGATG ATAAAGTCAC CAGTCAGCTG GCTACTGGAT TGAGGGTTTC
  551  TATCACCGAA CTGGAACGAA ATGGAGAGCT CGTCACCCTT ACTCAAGTCT
  601  TCGAAGAAGC ATCTACGGCC ATAAGCTGGA AGAAGGACAT TATGGGCGGA
  651  CGCCGAGAAG AACAGGAAGC AAGCAATGAC AAACTTAGTA GCAAATGAGG
  701  CCATCCTTAT GCGTGTGTAT TTTTGTGCGG AGGGATACTA TTAAGATTGC
  751  AGTTTCACCA AGTATAGCTT TTTATTTCAT TATAAGTTTC GTGTCAAAAT
  801  GTTTAAGCGA CCCGATCTCT CAGGCTGTTT TGCACGACTT TTCTGACTTT
  851  CCTCGCGTCT TTTTTCATGA AAATTGGATT ACCCGGAGTG ATGATTTTCT
  901  CACAGTGATT TTTCGTCCCC TTTTACAATA GCAAATGAAG CTGTTTTAGC
  951  AATATTTGTA GAAAGATATG TCACAAGAGG GCAGGCAAAA TGTCATACGG
 1001  AAGAGAAGAC ACTACGATTG AGCCCGACTT CATAGAACCA GATGCACCTT
 1051  TGGCTGCTTC CGGGGGTGTT GCTGACAACA TAGGCGGAAC TATGCAGAAT
 1101  TCAGGCAGCA GAGGGACGCT CGACGAGACT GTGCTGCAAA CACTAAAGCG
 1151  AGATGTGGTG GAGATTAATT CCAGACTGAA ACAAGTGGTA TACCCGCATT
 1201  TCCCCTCATT CTTTAGCCCC TCTGATGACG GGATAGGGGC GGCTGATAAC
 1251  GACATTTCAG CCAATTGCGA CCTGTGGGCG CCCCTTGCGT TTATCATATT
 1301  GTATTCTCTA TTTGTATCGC ATGCGCGGTC GCTGTTCTCG AGCCTATTTG
 1351  TGTCTAGTTG GTTCATTTTG CTGGTGATGG CATTGCATCT GAGACTCACC
 1401  AAGCCACACC AGAGGGTGTC GCTGATTTCG TACATCTCCA TTTCCGGGTA
 1451  TTGCTTATTC CCACAAGTGC TGAATGCCTT AGTCTCGCAG ATACTACTTC
 1501  CATTGGCCTA CCATATTGGA AAGCAAAATC GCTGGATTGT GAGGGTCCTG
 1551  TCGCTCGTGA AACTGGTGGT CATGGCGCTG TGCCTGATGT GGTCTGTGGC
 1601  CGCCGTTTCG TGGGTTACCA AGAGCAAGAC CATTATCGAG ATATACCTCT
 1651  GGCACTCTGT CTTTTTTGGC ATGGCTGGTT GTCAACTATT TTATAACACT
 1701  AGTTACATAT GTATAAAACC CAATATTCAT GGACATAGAA TTGCCTATCT
 1751  CGCGAGCCAC GGCAGAAAGT TCTGA
```

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FIGURE 73. *YGL198w* Protein Sequence

Nature 387:81-84 [97313265] (1997) The nucleotide sequence
of Saccharomyces cerevisiae chromosome VII.

Tettelin, H., Agostoni Carbone, M. L., Albermann, K.,
Albers, M., Arroyo, J., Backes, U., Barreiros, T., Bertani,
I., Bjourson, A. J., Bruckner, M., Bruschi, C. V.,
Carignani, G., Castagnoli, L., Cerdan, et al.

YGL198W Length: 261 March 31, 1999 10:05 Type: P Check:
1705 ..

```
    1   MSYGREDTTI EPDFIEPDAP LAASGGVADN IGGTMQNSGS RGTLDETVLQ
   51   TLKRDVVEIN SRLKQVVYPH FPSFFSPSDD GIGAADNDIS ANCDLWAPLA
  101   FIILYSLFVS HARSLFSSLF VSSWFILLVM ALHLRLTKPH QRVSLISYIS
  151   ISGYCLFPQV LNALVSQILL PLAYHIGKQN RWIVRVLSLV KLVVMALCLM
  201   WSVAAVSWVT KSKTIIEIYL WHSVFFGMAG CQLFYNTSYI CIKPNIHGHR
  251   IAYLASHGRK F
```
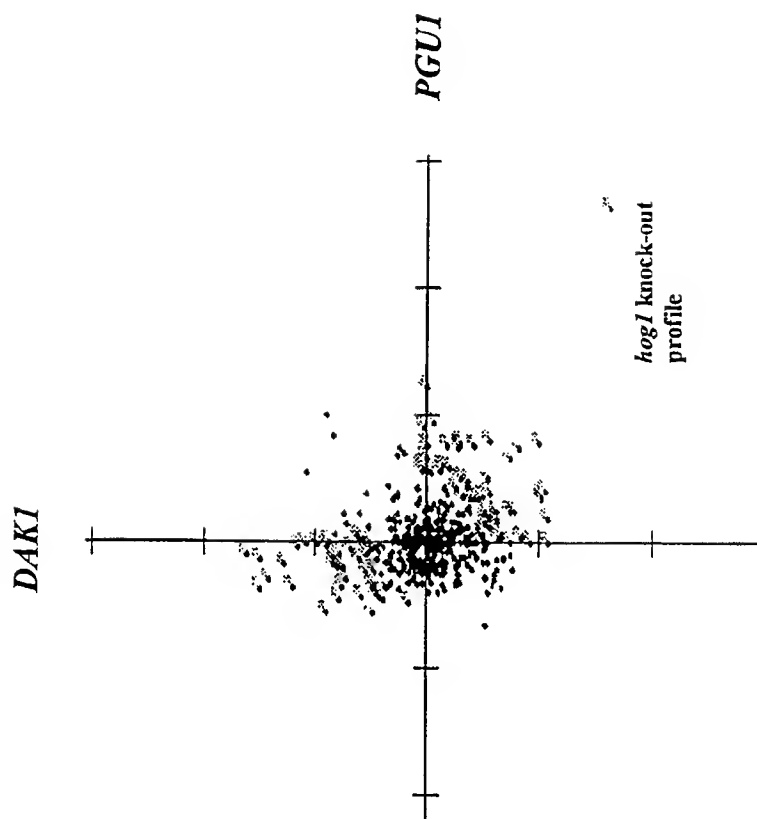
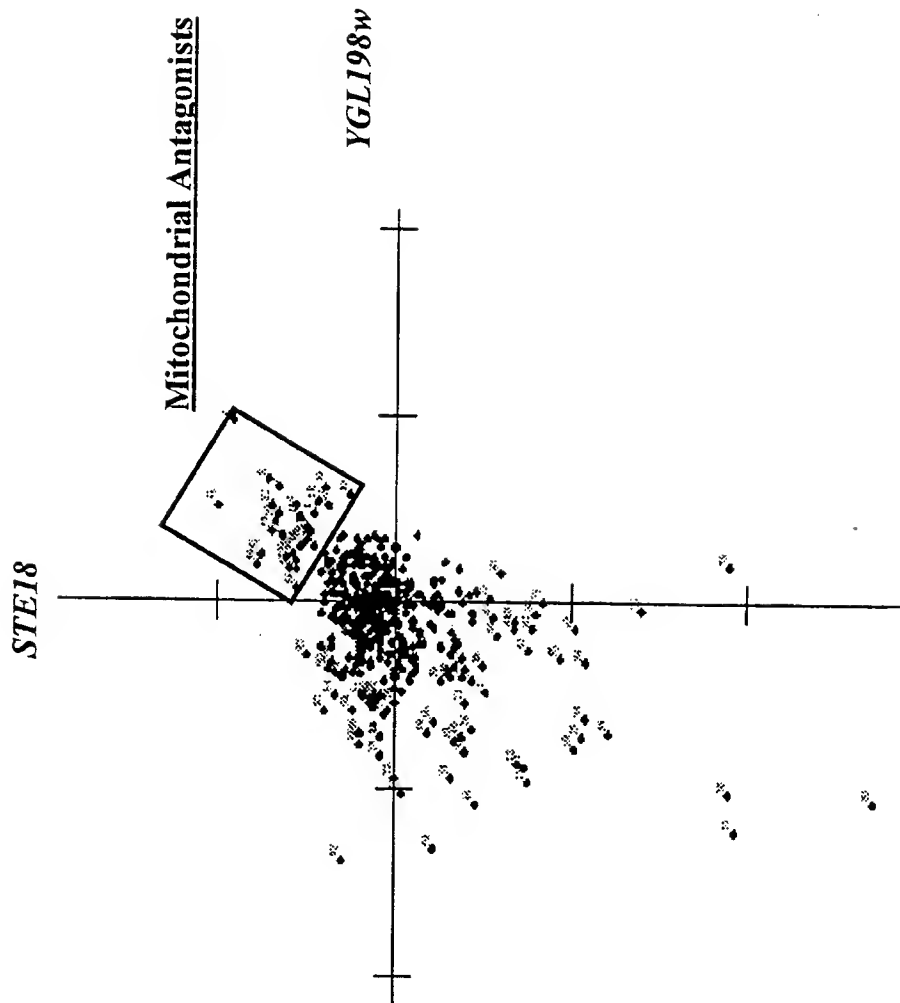Figure 74

Figure 75